

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., Editor in Chief
Medical Arts Building, Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS L. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.B.
University of Toronto Toronto

Clinical Pathology

W. C. MACCARTHY, M.D.
Mayo Clinic Rochester Minn.

Biochemistry

VICTOR C. MIFERS, Ph.D.
Western Reserve University Cleveland

Immunology

JOHN A. KOHLER, M.D.
University of Pennsylvania Philadelphia

Bacteriology

A. PARKER HITCHENS, M.D.
Bureau of Science Manila P. I.

Pathology

F. M. C. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD R. WELLS, M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR, M.D.
Mayo Clinic Rochester Minn.

Experimental Medicine

RUSSELL L. HADEN, M.D.
University of Kansas Rosedale

Surgery

DLAN LEWIS, M.D.
Johns Hopkins University Baltimore

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

VOLUME XII

OCTOBER, 1926—SEPTEMBER, 1927

ST. LOUIS
THE C. V. MOSBY COMPANY
1927

The Journal of Laboratory and Clinical Medicine

WAPPEN T. VALGHAN, M.D., Editor in Chief
Medical Arts Building
Pleasanton, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS F. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.D.
University of Toronto Toronto

W. C. MACCARTHY, M.D.
Javo Clinic Rochester Minn.

Biochemistry

VICTOR C. MYERS, Ph.D.
State University of Iowa Iowa City

Clinical Pathology

Pathology

HAUG WOOLFEY, M.D.
Los Angeles

Tuberculosis

IRALD P. WETZ, M.D.
Sanatorium Sanatorium Colorado Springs

HAROLD D. CATLOW, M.D.
Javo Clinic Rochester Minn.

Experimental Medicine

LESLIE L. HADY, M.D.
University of Kansas Roseville

Immunology

JOHN A. KOLMER, M.D.
University of Pennsylvania Philadelphia

ROBERT A. KILDUFF, M.D.
Atlantic City N. J.

Bacteriology

A. PARKER HITCHCOCK, M.D.
Bureau of Science Manila P. I.

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSS COMPANY 222 LOUISIANA BLVD. ST. LOUIS
Entered at the Post Office at St. Louis, Mo. as Second Class Matter.

LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment. Estimates Cheerfully Furnished Prompt Service

See our advertisements, pages 26 and 27

EDWARD P. DOLBEY & COMPANY - - PHILADELPHIA, PA.

[NUMBER THREE OF A SERIES]

Little Glimpses

OF THE SCIENTIFIC AND RESEARCH WORK
OF PARKE, DAVIS & COMPANY



Bio-Chemical Research

Just as the active principle of the suprarenal gland was isolated, it is hoped that, by further researches in the bio-chemical field, the active principles of other ductless glands may be discovered

PARKE, DAVIS & COMPANY

Home Offices and Laboratories

DETROIT, MICHIGAN

The Journal of Laboratory and Clinical Medicine

VOL. XII

ST. LOUIS, MO. OCTOBER 1926

No. 1

CLINICAL AND EXPERIMENTAL

ROUTINE SKIN TESTS IN CHRONIC NEPHRITIS*

By LOUIS C. P. BALDWIN, M.D., BOSTON, MASS.

IN VIEW of the lack of fundamental knowledge concerning the etiology of chronic nephritis, it seemed desirable to do skin tests on a series of patients with this condition in order to determine whether or not a certain proportion of them were hypersensitive to some particular food protein. The possibility that protein hypersensitiveness might be the basis of renal injury is suggested by certain experimental evidence. It has been shown for instance, by Longcope¹ that repeated subcutaneous shocking doses of horse serum or egg white in sensitized rabbits so spaced and of such an amount that they only caused mild symptoms, were capable of producing definite kidney lesions in a large proportion of the animals. His conclusions were later confirmed by Boughton.

Martin and Pettit,² whose work has been corroborated by Valerie Radot,⁴ obtained practically the same results by feeding their rabbits exclusively on powdered milk and meat protein. On this diet the animals lived from two weeks to five months, and in some cases developed a rapidly fatal acute nephritis, and in others a more chronic form. These investigators made no attempt, however, to determine why a high protein diet causes kidney injury, they neither described the symptoms in the course of their experiments nor apparently subjected their animals to subcutaneous doses of the offending protein, or in other manner attempted to determine the existence or non-existence of anaphylaxis.

Newburgh³ was also able to produce nephritis in rabbits by feeding them egg albumen, casein, and soy bean. Unquestionably, therefore, a high protein diet causes kidney damage in rabbits but the mechanism by which this is

*From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Massachusetts.
Received for publication March 26 1926

produced is still obscure. In animals fed on large amounts of egg albumen, Newburgh was unable to demonstrate precipitins in the blood for the ingested protein, consequently it would not appear that anaphylaxis was responsible for the nephritis in his animals.

Some more recent work of Bell and Hartzell⁶ points out certain pitfalls one is in danger of when too close analogies are drawn between what occurs in laboratory animals under experimental conditions and what is found in man. They maintain in the first place that it is very difficult to be certain that experimental procedures are alone responsible for kidney lesions, and in the second place that a comparison is still further subject to question in view of the fact that there are marked differences between the lesions found in rabbits which have been subjected to repeated nonfatal poisoning with foreign protein and those found in man. In these animals, moreover, disease of the kidney is quite common and often of spontaneous occurrence, that is to say, independent of any intentional procedure carried out by the investigator. They conclude that there is no experimental evidence that foreign protein is in any way responsible for chronic nephritis in man.

In spite of their evidence to the contrary, it does not seem unlikely that hypersensitiveness may be at the bottom of certain cases of chronic nephritis in which no other ascertainable cause can be found. This applies particularly to those patients who present no antecedent acute infections and no histories of repeated acute exacerbations of their nephritis. Presumably in this type of case, sensitization resulting in renal injury might occur through the gastrointestinal tract. That proteins may, under certain conditions, be absorbed unchanged after ingestion, has been pretty definitely established by Schloss and Worthen,⁷ Lust,⁸ Moro,⁹ and others, who have shown the presence of unaltered food proteins in the blood of some infants. It is not conceivable that absorption of this kind might account for the manner in which some children become hypersensitive to proteins in their diet, though possibly sensitization through the gastrointestinal tract does not always account for hypersensitiveness, since there are exclusively breast-fed infants with eczema who give positive skin reactions with egg albumen, cow's milk or other proteins which they have never eaten. In order to explain sensitization on similar grounds in these cases one would have to assume that unaltered proteins were absorbed by the mother and excreted unchanged in her milk, so that the nursing infant could obtain the offending protein from that source. This point has been investigated by Shannon,¹⁰ who claims to have shown the presence of food proteins in mother's milk by anaphylactic experiments on guinea pigs. Stuart,¹¹ however, was unable to verify his experiments in spite of the fact that he used more delicate methods for identifying the presence of food protein.

Granting that an individual is hypersensitive we are not vitally concerned in this investigation with how that condition was brought about. We know that foods are capable of causing symptoms of intoxication upon ingestion in hypersensitive individuals. An extreme case recently cited by Coca¹² is illustrative of this fact. In this individual swelling of the lips and tongue followed by a generalized pruritus would occur when cooked pea was taken into the mouth without being swallowed.

SELECTION OF CASES

The present series consists of 23 unselected cases of chronic nephritis. At first it was thought advisable to exclude the patients who gave a definite history of an acute infection immediately anteceding their nephritis because of the fact that in this type of case the renal injury seemed to be bacterial in origin. More careful analysis of their histories, however, made it apparent that one could never be sure in an individual instance that the infection was directly responsible for the nephritis. On the contrary, might one not be dealing with an acute exacerbation of a chronic process? In other words, was it not reasonable to suppose, that a preexisting condition was only being stirred up by the bacterial toxemia and not initiated by it? In favor of this view is the fact that several of the cases gave no histories of an antecedent infection, and yet the course of their disease was interrupted in its progress by acute exacerbations sometimes directly following an infection, at others apparently spontaneous in origin.

In addition to their renal history the patients were carefully questioned as to whether they, or any members of their family, had had asthma, hay fever, urticaria, or chronic bronchitis. This was done for two purposes, to exclude the possible independent existence of two diseases in the same person, and to determine if protein idiosyncrasies are more common in chronic nephritis than in normal individuals. One would expect that if nephritis were often caused by sensitization to protein, that some of the more generally recognized symptoms of protein sensitization would manifest themselves at some time in a certain proportion of the patients.

Each patient was further questioned as to whether there was anything of a dietary nature which he avoided either because of an actual distaste or because it disagreed with him. This is an important symptom of intolerance to protein, particularly in children. One commonly finds that a mother has been unable to make her child eat eggs or drink milk, and subsequent investigation shows that the child is hypersensitive to the protein for which he has an active dislike and consequently refuses to eat.

SUMMARY OF CASES

CASE 1—N. C., aged thirty eight years. In October, 1918, had influenza, followed shortly by swelling of the extremities, headaches, dyspnea, weakness, and edema of the eyelids. Course very gradually progressive. Since 1919 urine showed a fixed low specific gravity, a heavy trace of albumin, moderate number of hyaline and granular casts. Blood pressure and BUN gradually rose, phthalein dropped. Developed a terminal pericarditis. No history of protein sensitization.

CASE 2—P. M., aged fifty two years. Repeated attacks of acute articular rheumatism and erysipelas of face on several occasions, in 1917, acute nephritis immediately followed erysipelas. Since 1915 had had nocturia once. The functional tests showed a persistent nephritis with latterly a blood pressure between 140/84-156/98, phthalein between 34 and 48. The urine showed numerous casts and a fixed low specific gravity. No history of protein sensitization.

CASE 3—H. C. B., aged thirty four years, had scarlet fever in infancy. In 1916 had acute tonsillitis followed by nephritis. Suffered from severe headaches and weakness since 1917 when urine examination showed constant albumin and casts. Course gradually progressive. Latterly blood pressure over 200 systolic, phthalein below 20 per cent, BUN

slightly elevated. Noteworthy persistence of red blood cells in the urine. No history of protein sensitization.

CASE 4—H G B, aged twenty three years. Following influenza in 1919 developed symptoms of acute nephritis. In 1921 because of persistent haematuria a double renal de-capsulation performed without benefit. Renal functional tests persistently very slightly impaired. Haematuria persists with occasional periods of acute exacerbation. Has always disliked eggs. Eight months previous to having skin tests, had hives of two days' duration. No other history of protein sensitization.

CASE 5—E W, aged twenty six years, has had numerous head colds for years. Much infection around the teeth since childhood. In 1917 complained of a gradual onset extending over a period of months, of failing vision, headache, and edema. At this time there was a well marked albuminuric retinitis. The blood pressure was 240/140 and the urine showed a trace of albumin and an occasional hyaline cast. Since then the course has been steadily, but very slowly progressive, lately complicated by an acute pericarditis. No history of protein sensitization.

CASE 6—A R H, aged nineteen years, had several attacks of tonsillitis in childhood. In 1919, without known cause, puffiness of face and edema of lower extremities developed. Six months later had a mild attack of influenza followed by an acute nephritis. In 1921 another acute attack with general anasarca, edema of retina and discs, urinary retention, moderately elevated blood pressure, but good renal function. One month later had a similar but more severe attack with good renal function but with convulsions, Cheyne Stokes respiration, and coma. Blood pressure at times 220/154. Numerous hemorrhages occurred in both eyes. Improvement set in gradually. Since then the progress has been steadily downhill, with a slow drop in renal function. No history of protein sensitization.

CASE 7—W J D, aged sixty years, has had dyspnea, palpitations of the heart, and precordial pain on exertion for a number of years. Physical examination revealed an enlarged heart, accentuated A₂, and a blood pressure of 162/103. Phthalein elimination slightly impaired. Urine showed a very slight trace of albumin and rare granular casts. No history of protein sensitization.

CASE 8—R A, aged twenty seven years. History of nephritis for ten months. Acute symptoms developed immediately following an extensive, reddened, scaly eruption of the face. Course rapidly downhill with a progressive impairment in renal function. No history of protein sensitization.

CASE 9—B M, had a chronic cough for years. Tonsillitis and scarlet fever in childhood. Following the scarlet fever had dizziness, blurred vision and nausea, also edema of the lower extremities. Since primary attack these symptoms have recurred at intervals and for several years the patient has had nocturia. Had convulsions during two pregnancies, which had to be terminated. Renal function not depressed. Urine showed moderate to numerous hyaline casts, some with fat, rare to moderate red blood cells. No history of protein sensitization.

CASE 10—H B D, aged twenty years, had diphtheria eight years ago. For seven years has had headaches and nose bleeds. Four years before noticed puffiness of the eyelids. Lately has felt tired and weak. Except for slight edema, physical examination negative. Renal functional tests normal. No elevation of blood pressure. Urine shows slight trace of albumin casts, and persistent red blood cells. No history of protein sensitization.

CASE 11—H B, aged nineteen years, has had no past illnesses. Six months before began to suffer from malaise and a general tired feeling. Three and one half months before edema of eyelids appeared. Aside from large tonsils and edema of eyelids and lower extremities, physical examination essentially negative. Renal function very slightly depressed at times. Blood pressure occasionally a trifle elevated. The urine showed a trace to a large trace of albumin, numerous fatty and granular casts, rare to numerous red blood cells. No history of protein sensitization.

CASE 12—A L H, aged thirty eight years. Acute nephritis a year before following pneumonia. Since then the urine has persisted to show albumin, casts and red blood cells. Renal function is good, blood pressure slightly elevated at times. No history of protein sensitization.

CASE 13—I M, aged fourteen years. Pneumonia twice once in infancy and once three years before. Tonsillitis several times the last attack quite recently. Patient felt well until a year and a half before when without apparent cause, she noted general weakness and shortness of breath on exertion. In September of the same year, following a severe cold, her face became very puffy. There were no other symptoms of nephritis. Examination showed a general anæmia with secondary anemia. The urine contained albumin, casts of all descriptions and moderate to numerous red blood corpuscles. The blood pressure was 140/100. There was no impairment in renal function. The course has remained stationary except for the fact that there have been periods in which the edema has entirely subsided. No history of protein sensitization.

CASE 14—M B, aged thirty four years. Diphtheria at two years. A year before began having swelling of ankles which soon spread upward to the legs and thighs. Except for moderate shortness of breath on exertion felt quite well. The urine showed albumin and casts. Renal functional tests showed a slight impairment at first but none lately. There was no elevation in blood pressure. Upon an Epstein diet the edema almost entirely cleared up and patient felt much better. No history of protein sensitization.

CASE 15—S S, aged forty nine years. Slight palpitation of the heart and dyspnea on exertion swelling of the leg and nocturia for three years. Seven months before admission to hospital began to suffer from dizziness blurred vision nervousness and insomnia. This was at the time of her last menstrual period. Physical examination showed a moderately enlarged heart with a blowing apical systolic murmur slight pitting edema of the limbs and sclerosed vessels. The blood pressure was 205/120. There was no impairment in renal function, and the urine was negative. This apparently is a case of vascular hypertension and not of nephritis. No history of protein sensitization.

CASE 16—F T, aged thirty years. Diphtheria at six years of age mild influenza in 1918. In June 1920, had feet strapped to support fallen arches. Noticed at this time swelling of feet and ankles. No other symptoms. The renal functional tests remained normal at all times. There was no elevation of blood pressure. The urine showed considerable albumin, hyaline and granular casts, some fatty casts, free fat and red blood cells. Course on the whole was stationary. Lately there has been a recurrence of edema. No history of protein sensitization.

CASE 17—T W B, aged thirty two years has had asthma for fifteen years also for several years hay fever coming on in September and remaining until the first frost. Gradual onset seven months before with general weakness and mild edema of the ankles. No antecedent infections. Blood pressure and renal functional tests normal. The urine showed a persistent trace of albumin, numerous hyaline casts, rare to moderate granular and few fatty casts, also rare to numerous red blood cells and white blood cells.

CASE 18—H C, aged twelve years, repeated attacks of sore throat. One and one half years ago suddenly developed edema of the face and lower extremities. Vomited for a week and had several convulsions. At this time the urine showed numerous granular and hyaline casts and was of low fixed specific gravity, the blood pressure was 200/120. A year later the child had an acute attack of tonsillitis accompanied by renewed signs of active destruction of the kidneys. The course has been very gradually progressive. No history of protein intolerance.

CASE 19—S C, aged forty five years always well up to fourteen months before, when two to three days following an attack of grip hematuria and swelling of the lower extremities were noticed. Upon rest in bed the swelling and hematuria gradually disappeared. She resumed her housework and felt quite well until three months before when

the swelling of her lower extremities returned, and her abdomen began to enlarge. The blood pressure was 162/100, the phthalein 10 per cent, the urine showed a trace of albumin and numerous hyaline and granular casts. No history of protein sensitization.

CASE 20—J P, aged forty years. Measles and smallpox early in life. Two years before, patient caught cold while bathing and developed a severe, productive cough. At the same time became short of breath on exertion, this persisted until six weeks before admission to the hospital when, in addition to the cough, she complained of a loss of appetite and swelling of the feet. For two years she had had nocturia. The urine persistently showed signs of active kidney destruction. Her condition grew rapidly and progressively worse. She died in uremic coma. No history of protein sensitization.

CASE 21—C H, aged twenty five years. Diphtheria in childhood, and tonsillitis every winter until tonsillectomy three years before. Thirteen months before admission, during sixth pregnancy, began having failing vision and suboccipital headaches. Two months later convulsions set in, and patient had a premature labor. Was better for a time then gradually got worse. BUN increased, phthalein depressed. Urine showed a large trace of albumin and numerous casts of all descriptions. No history of protein sensitization.

CASE 22—M C, aged forty three years. Childhood diseases, including scarlet fever. During pregnancy fifteen years before had general anasarca, headache, marked albuminuria. Another pregnancy six years before resulted in a miscarriage. Has had nocturia 1 to 3 times for fifteen years. Also mild, occasional puffiness of the ankles and frequent headaches for fifteen years. For two years has been short of breath upon the slightest exertion, and for seven months her eyesight has been failing. Retinal examination revealed a marked albuminuric retinitis. The BUN was normal, the phthalein slightly depressed. The urine showed a large trace of albumin and numerous hyaline and granular casts. No history of protein sensitization.

CASE 23—J P M, aged forty eight years. Occasional mild sore throat for years, and measles and typhoid in childhood. Was examined and passed for life insurance ten years before. One year before began having nocturia 2 to 4 times, otherwise felt perfectly well. Four months before, was refused an increase in his life insurance on account of albumin in the urine. Three weeks before began having trouble with his eyes, the sight failing rapidly since. Physical examination showed marked arteriosclerosis with hypertension, and a severe retinitis. Renal functional tests depressed. The urine showed considerable albumin, hyaline and granular casts, and red blood cells. No history of protein sensitization.

PROTEINS EMPLOYED FOR THE SKIN TESTS

Only the more common food proteins were employed for skin tests, since it seemed reasonable to assume that if chronic nephritis were the result of sensitization of dietary origin, substances which were frequently eaten could alone be held responsible for the renal injury, for it would be difficult to conceive how a condition, presumably dependent on repeated protein reactions could be caused by a protein only rarely eaten.

The same proteins were employed for the skin tests in every case. They were applied cutaneously by the Walker technic. That is to say, small cuts were made in the skin of the forearm, just deep enough to penetrate the superficial layers but not to draw blood. A protein in powdered form was placed on each of these cuts except one, and a drop of decinormal sodium hydroxide added to act as solvent. From time to time the sites were moistened with the sodium hydroxide, and at the end of half an hour the proteins were washed off and readings made. For control a cut was made, upon which decinormal sodium hydroxide alone was placed. In interpreting re-

sults, a positive reaction was considered as one consisting of a wheal, at least 0.5 cm in diameter, surrounded by a variable zone of erythema, while if there were only a zone of erythema of from 15 to 20 mm in diameter, but no wheal, the reaction was termed doubtful. Of course, a comparison with the control site is important in every case, but particularly in cases of doubtful reactions since the latter resemble closely nonspecific irritative phenomena. Consequently, whenever a doubtful reaction was obtained it was repeated on a fresh site the same day and on a subsequent day. Only if the reaction was repeatedly obtained was it recorded as of possible significance. The tested sites were again inspected at the end of twenty-four hours for the possible development of delayed reactions.

FOOD PROTEINS EMPLOYED CUTANEOUSLY

1 Wheat globulin	7 Rice	13 Lactalbumen	19 Chicken
2 Wheat glutenin	8 Potato	14 Casein	20 Halibut
3 Wheat gliadin	9 Pea	15 Cocoa	21 Codfish
4 Oat	10 Bean	16 Beet	22 Salmon
5 Barley	11 Tomato	17 Lamb	
6 Egg	12 Egg white	18 Pork	

Since a number of the patients had suffered from repeated attacks of tonsillitis or had infected teeth it was thought advisable to test them also with bacterial vaccines of the more common mouth organisms with the view that possibly they might show a sensitization to some bacterial protein. For this purpose the more delicate intracutaneous method was employed because the vaccines which we used for the purpose were fairly dilute when compared with the proteins applied in powdered form, and consequently it was thought that a mild positive reaction might appear by this method when the less delicate cutaneous application would fail to react. Briefly the arm was cleansed with alcohol and about 0.05 cc of the vaccine was injected intradermally with a very fine needle. The size of the initial wheal was noted, and a final reading was made at the end of half an hour and again in twenty-four hours. When an autogenous vaccine had been made from the patient's tonsils or infected tooth socket a skin test was also performed with the latter.

VACCINES EMPLOYED FOR INTRADERMAL TESTS

<i>Streptococcus pyogenes</i>	<i>Streptococcus mitis</i>	<i>Streptococcus fecalis</i>
<i>Streptococcus infrequens</i>	<i>Streptococcus salivarius</i>	<i>Staphylococcus aureus</i>
<i>Streptococcus anginosus</i>		

RESULTS

The accompanying chart shows that only two cases gave definitely positive reactions to food proteins. Case 4 had been unable to eat eggs from childhood because he disliked them and because he had learned from experience that they disagreed with him, causing considerable epigastric distress. On one occasion, a year previous to doing the skin tests he had had a mild attack of urticaria lasting two days but otherwise his history was negative regarding protein hypersensitiveness. There was a mild reaction to egg white in this case. Upon reviewing the history of his nephritis, one sees that the onset was sudden, following an attack of influenza, and the subsequent course

CHART I

PATIENT	PREVIOUS ILLNESS	ANTECEDENT INFECTION	DURATION	HISTORY OF PROTEIN ALLERGY	FOOD PROTEINS		BACTERIAL VACCINES		AUTOGENOUS VACCINE
					POSITIVE	DOUBTFUL	POSITIVE	DOUBTFUL	
1	influenza	influenza	4 yr	none	0	0	0	0	
2	rheumatic fever, erysipelas	erysipelas	5 yr	none	0	0	not done	0	
3	scarlet fever, tonsillitis	tonsillitis	5 yr	none	0	0	0	Staph aureus	0
4	influenza	influenza	3 yr	always disliked eggs Hives on one occasion for two days	egg white +	0	0	Staph aureus	
5	numerous head colds, much infection around teeth	none	5 yr	none	0	0	0	Staph aureus	
6	repeated tonsillitis in childhood	none	3 yr	none	0	0	0	0	
7	negative	none	5 1/2 yr	none	0	0	not done	0	
8	eczema of face	none	1 yr	none	0	0	0	0	
9	chronic cough, tonsillitis, scarlet fever	scarlet fever	15 yr	none	0	0	0	Staph aureus	
10	diphtheria	possibly diphtheria	7 yr	none	0	rice	0	0	0
11	none	none	8 mo	none	0	0	0	Strep infrequens	0
12	pneumonia	pneumonia	2 yr	none	0	0	0	0	
13	pneumonia twice, tonsillitis several times	severe cold	2 yr	none	0	rye	0	0	
14	diphtheria	none	1 1/2 yr	none	0	0	0	Staph aureus	
15	none	none	1 yr	none	0	0	not done	0	
16	diphtheria	none	2 yr	none	0	pea	0	0	
17	asthma	none	9 mo	asthma 15 years hay fever for years	barley ++, oat +, wheat gluten + rye +, rye weed +, wheat gluten + wheat globulin +	0	0	0	0
18	repeated tonsillitis	none	1 1/2 yr	none	0	0	not done	0	
19	negative	influenza	1 1/2 mo	none	0	0	not done	0	
20	measles small pox	cold	2 yr	none	0	0	0	0	
21	diphtheria, frequent tonsillitis	none	10 mo	none	0	0	0	0	
22	scarlet fever	none	15 yr	none	0	barley	0	0	
23	typhoid, occasional sore throat	none	1 yr	none	0	0	0	0	

is rather remarkable in showing a persistent fairly severe hematuria. An intracutaneous test with *Staphylococcus aureus* gave a doubtful reaction. In Case 7, a man of thirty two years of age there was a history of asthma of fifteen years standing. This patient claimed that his first attacks occurred while working in a stable, and were accompanied by sneezing, running of the eyes and nose. At first his attacks were closely associated with stable work though the patient was inclined to blame oats rather than horses for his condition, since the handling of oats always brought on an attack. Two years after his onset his asthma occurred at any time, but particularly at night and during damp weather. For several years he has also had hay fever, coming on regularly in September and lasting until cold weather. Cutaneous tests in this case were strongly positive with barley, oat, rye, and somewhat less strongly with wheat and ragweed. The nephritis was of gradual onset and of only seven months duration. These two cases were the only ones that gave histories suggesting hypersensitiveness to food proteins and they were the only ones that gave positive reactions with food proteins. Four patients gave doubtful reactions. Case 10 to rice, Case 13 to rye, Case 16 to pea and Case 22 to barley.

Intracutaneous tests with the bacterial proteins were entirely unsatisfactory. Of the 18 cases tested none gave positive results. There were 6 cases in which the reactions were doubtful, 5 of these with *Staphylococcus aureus*, and 1 with *Streptococcus infrequens*. Four cases were tested with their autogenous vaccines, but even with the bacterial vaccines obtained from their teeth or tonsils negative results were obtained. Case 3 gave a doubtful reaction with *Staphylococcus aureus* but negative with his autogenous vaccine which were respectively, *Streptococcus mitis* from an infected tooth and *Streptococcus pyogenes* from the tonsils. Case 11 reacted doubtfully with *Streptococcus infrequens*, but gave no reaction with his autogenous vaccine, which was a *Streptococcus fecalis* recovered from the tonsils.

CONCLUSIONS

From this series one can draw no conclusions. If the skin test is an accurate index of protein sensitization it would seem that chronic nephritis is not commonly caused by proteins derived from the food. But on the other hand, there is abundant evidence elsewhere in the literature to show that protein sensitization may exist in spite of repeated negative skin tests, and therefore this problem will have to be studied further before any decision can be made.

REFERENCES

- ¹Longcope Jour Exper Med 1913 xvin, 678, 1915, xxii, 793
- ²Boughton Jour Immunol, 1916, i, 105
- ³Martin and Pettit Ann de l'Inst Pasteur, 1913, vii, 532
- ⁴Valerie Radot Societe de Biologie, 1914
- ⁵Newburgh Arch Int Med, 1919, xxiv, 359
- ⁶Bell and Hartzell Jour Infect Dis, 1919, xxiv, 618
- ⁷Schloss and Worthen Am Jour Dis Child, 1916, xi, 342
- ⁸Lust Jahrb f Kinderh, 1913, lxxvii, 244
- ⁹Moro Munchen Med Wchnschr, 1900, 49
- ¹⁰Shannon Am Jour Dis Child, 1921, xxii, 223
- ¹¹Stuart Am Jour Dis Child, 1923, xxv, 135
- ¹²Coca and Grovo Jour Immunol, 1925, x, 445

BLOOD URIC ACID IN NEPHRITIS*

BY WILLIAM PAUL HOLBROOK, M D, AND HOWARD DAVIS HASKINS, M D,
PORTLAND, OREGON

THE aim of the work reported in this paper was to determine the clinical value of estimations of the uric acid of the blood as an indication of kidney impairment. For this purpose a study was made of 138 hospital cases, including estimations of urea, creatinin, uric acid and hemoglobin of blood, urine examination, functional tests and blood pressure, in addition to noting the age, history and clinical observations. Fifty-one of these cases were not used in preparing this paper either because they proved to be nonnephritic or because the nephritis was complicated by other conditions.

We have been interested in uric acid determinations for several years, chiefly because of the numerous statements in the literature that uric acid is excreted by the kidney with greater difficulty than urea or creatinin, and that, therefore, its retention gives the earliest indication of renal impairment. Myers, Fine and Lough¹ state that uric acid retention occurs before urea or creatinin are increased. Baumann, Hansman, Davis and Stevens² believe that uric acid determination is probably the most delicate index of renal function. Upham and Higley³ claim that uric acid is the most difficult of all the waste products to excrete. Myers and Kilham⁴ also confirm that opinion.

We suspected that the current methods of estimating uric acid might be unsatisfactory, so we made a careful study of this question first. This work is reported in a recent paper.⁵ We compared the results on 55 normal blood specimens by three methods, namely, the silver lactate and zinc chloride precipitation methods and the direct method. None of the methods were entirely satisfactory for a uric acid content of 1.5 to 3.7 mg per 100 cc of blood, the direct method being the worst of the three. We were able, however, to introduce a modification into each of the methods so that they yielded good results. On trying these three modified methods with 25 hospital bloods, as well as with normal bloods, we secured very good results that show little variation by the different methods. Having determined how to secure accurate uric acid estimations, we were in a better position to make an intelligent study of the clinical value of such estimations.

After eliminating all cases in which the blood chemistry findings might be due in part to some condition besides a definite kidney lesion, there remain 87 cases of nephritis of various types. No cases are included in which metabolic disorders play any part. A summary of the results arranged in the order of the urea nitrogen content of the blood is given in Table I. Most of the results (i e., up to 96 mg urea N) are shown graphically in Chart 1, enabling one to

*From the Department of Biochemistry, University of Oregon Medical School, Portland, Oregon.

Received for publication April 18 1926

see at a glance the comparative level of the uric acid, creatinin and urea nitrogen content of the blood in these cases. The line drawn at 16 mg urea N, is the dividing line between normal figures and figures indicating retention. This line represents 4 mg of uric acid and 16 mg of creatinin.

DISCUSSION

The 12 cases that show no retention consisted of (1) cases of focal embolic nephritis (blood and casts in the urine, and a definite focus of infection), (2) several cases of early acute nephritis and (3) cases of hypertension (trace of albumin and occasional casts in the urine).

TABLE I
UREA NITROGEN, URIC ACID AND CREATININ CONTENT OF NEPHRITIC BLOODS

NUMBER OF CASES	RANGE OF UREA N AS MG	RANGE OF URIC ACID MG	RANGE OF CREATININ MG	NO CASES HAVING PATHOL UREA N	NO CASES HAVING PATHOL URIC ACID	NO CASES HAVING NORMAL URIC ACID	NO CASES HAVING PATHOL CREATININ	NO CASES HAVING NORMAL CREATININ
12	8-15	12-33	12-14	none	none	12	none	12
26	16-24	17-40	12-23	26	none	26	6	20
29	25-50	15-54	13-31	29	9	20	26	3
20	51-367	22-133	22-99	20	17	3	20	none
87				75	26		52	

Urea retention in 86 per cent of the cases. Creatinin retention in 60 per cent of the cases. Uric acid retention in 30 per cent of the cases.

In the first group of cases that show pathologic findings the urea N content is from 16 to 24 mg, which is the range of early retention. Not a single one of the 26 cases shows uric acid retention. In the next group which comprises the cases showing moderate retention, only 9 out of the 29 had uric acid retention in addition to the urea retention. In the group of marked retention (51 to 367 mg urea N) 3 of the uric acid findings were normal. In these 3 cases with normal uric acid content the lowest creatinin estimation was 27 mg and the lowest urea N was 62 mg. This failure of uric acid retention when marked retention was indicated by the content of other substances, is difficult to understand, except possibly on the supposition of diminished production of uric acid.

On the other hand, there was retention of creatinin in 46 of the 49 cases in the last two groups (25 mg or more of urea N). The 3 cases having normal creatinin gave urea N estimations below 30 mg. The general tendency toward increase of creatinin paralleling distinct increase of urea N is well shown in Chart 1.

Summarizing the 87 cases of nephritis 86 per cent showed urea retention 60 per cent showed creatinin retention and only 30 per cent showed uric acid retention. The latter results are inconsistent with the idea that uric acid is the first substance to be retained in consequence of damage to the kidneys. In our series of cases uric acid estimations have given no information of diagnostic or prognostic value that was not given in a more reliable way by the urea and creatinin estimations.

As has been pointed out by others creatinin estimations are valuable in certain cases as an aid to prognosis. Thirteen of our cases gave creatinin estimations of 49 mg or more. Twelve of these have died. The remaining one has

recovered and shows no retention. It was in all probability a case of sympathetic anuria following nephrectomy. The retention disappeared in a few days.

We consider creatinin estimation valuable also as a check on urea estimation, particularly in cases of marked retention. The curve for creatinin content runs roughly parallel to the urea curve (above 25 mg urea N), as shown by Chart 1. In contrast with this point it will be noticed that the curve for uric acid shows no relation whatever to the other curves.

Estimations of sodium chloride were made in all the cases, but we do not report them because we have been unable to attach any significance to them. The

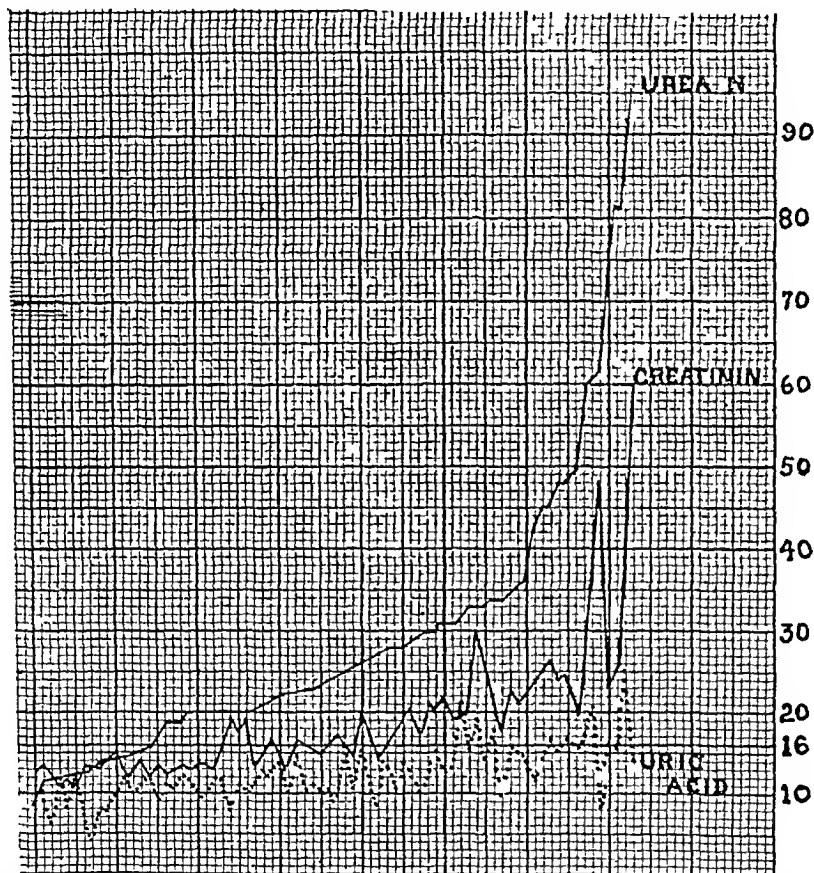


Chart 1—Curves for the Creatinin Uric Acid and Urea N Content of Nephritic Bloods. The line dividing normal and pathologic findings represents 16 mg urea N, 4 mg uric acid and 16 mg creatinin. The urea content at any particular point on the uppermost curve can be read by referring to the figures in the margin. To read the creatinin content from the middle curve the marginal figure must be divided by 10. The uric acid content is found by dividing the marginal figure by 4.

estimations seemed to be of no clinical value until they rose to nearly 600 mg per 100 cc of blood. Very few of the cases gave an estimation as high as that even with marked retention of urea and creatinin. No difference in chloride content was observed in cases with edema and without edema.

The question of the range of normal findings for a particular constituent of blood is quite vital in blood chemistry work. From a large series of estima-

tions we conclude that urea N normals run from 7 to 15 mg per 100 cc of blood, and that more estimations are near 8 mg than are near 14 mg. All results above 15 mg seem to us to call for attention clinically. In a previous paper⁵ we reported the range of uric acid in 55 normal persons as 1.5 to 3.7 mg, but it is probable that the results are not clinically significant until 4 mg is reached. The range of creatinin normals is in our opinion much lower than is ordinarily stated, probably 1 to 1.6 mg. We have not secured an estimation above 1.6 mg in normal or in nonnephritic individuals. We found a wide variation of sodium chloride content in normal bloods running from 316 to 550 mg per 100 cc.

Attention has been called to the possible effect of diet (exogenous purins) on the uric acid content of the blood. This was not a factor in the cases reported in this paper, since all of the 87 nephritics were in the hospital on a low protein diet.

It may be asked, how was it that others secured pathologically significant uric acid estimations in the earliest stage of retention while we had only one such estimation in all the cases showing less than 30 mg of urea N? The only suggestion that occurs to us is that all of that previous work was done before the observation was made that starvation⁶ results in distinct increase of blood uric acid. It is apparent now that there is a relationship between ketosis and increased uric acid content of the blood. This has been emphasized in a recent paper⁷ on the effect of high fat diets. We consider it possible therefore, that the previous uric acid findings, which were interpreted as indicating retention, were really due to dietary factors that were not recognized at the time.

Methods—It seemed desirable to make all estimations on the protein free filtrate prepared by Folin's tungstic acid method. A smaller amount of blood suffices for carrying out the methods. It gives greater uniformity in handling bloods, also greater leeway in doing the laboratory work because the filtrate keeps satisfactorily when saturated with toluol.

Urea N estimation—Our standard method consists in warming 4 cc of oxalated whole blood mixed with urease and buffer phosphate for thirty minutes adding potassium carbonate reagent and by aeration drawing the ammonia over into 25 cc of N/70 acid. After titration of the acid left unneutralized, the urea N is easily calculated. This method must be started while the blood is still fresh. It was difficult at times to find opportunity to run the estimation promptly, also in some cases 4 cc of blood could not be spared.

Folin⁸ suggests a method using a small amount of blood filtrate in which the final step is nesslerization. Never having been convinced that nesslerization and colorimetric estimations of ammonia are desirable or necessary, we have changed Folin's method so as to permit of titration.

Technic—Measure 10 cc of blood filtrate into a large pyrex test tube and add 1 cc each of urease solution and buffer solution. Warm in a water bath at about 55° C for fifteen minutes. Add 5 drops of caprylic alcohol, two glass beads, and 2 cc of saturated borax solution. Connect at once with the condenser (we use a Hopkins' bulb fitted to the pyrex tube with a rubber stopper and a 25 cc pipette as a delivery tube attached to the free end of the bulb).

Add 5 cc of N/70 acid, 5 cc of water and 1 cc of 0.05 per cent sodium alizarin sulphonate solution to a short test tube (1 inch diameter), and then adjust the tip of the apparatus so that it is in the liquid. Heat with a small flame from a microburner. About four minutes' boiling is generally required, but continue the distillation for a full minute after steam comes over. During the last half minute keep the tip of the delivery tube raised slightly above the acid solution (we lower the test tube by the use of blocks). Rinse off the tip with a little distilled water. Titrate the distillate mixture with N/350 sodium hydroxide.

Calculation—Subtract the cc used for titration from the titration figure for the control (run this control once a day using urease and all reagents but no filtrate), then multiply by 4, the result is milligrams of urea N in 100 cc of blood.

Reagents—(1) Urease solution—grind up a tablet of urease (0.1 gm) in a mortar and mix thoroughly with 5 cc of water. (2) Buffer solution—dissolve 2.8 gm of sodium pyrophosphate in 100 cc of N/10 phosphoric acid. (3) N/350 sodium hydroxide—prepare a stock solution of N/35 NaOH and keep it in a nonsol or pyrex flask from this prepare each week a supply of N/350 solution by making a 1 in 10 dilution.

Note—The method was checked against the standard whole blood method by running both methods on the first 34 cases (urea N varying from 8 to 200 mg). The variation in the results by the two methods was small, and in many cases they agreed as closely as duplicates by one method. The filtrate method was, therefore, adopted for the rest of the cases, checked occasionally by estimation by the standard method.

Uric acid estimation—The direct method (Folin-Benedict) as modified by us⁸ was used. It is necessary to repeat the warning given in our previous paper that the direct method as ordinarily carried out is very unsatisfactory when the concentration of uric acid is below 4 mg. With our modification, however, which consists in adding 0.5 cc of pure uric acid solution (equivalent to adding 2 mg of uric acid to 100 cc of blood) to 5 cc of blood filtrate before adding Folin's sodium cyanide and phosphotungstic reagents, the color that is developed is satisfactory for estimation, and the results are quite accurate. When there is a variation from the check method (precipitation by silver or zinc) it is generally on the side of slight excess. For research accuracy a check method should be run, we believe, whenever the direct method gives results above the normal content of the blood.

Recently Benedict⁹ and Hunter and Eagles¹⁰ have reported the isolation of a new substance from blood which gives a blue color with the uric acid reagents. This compound is supposed to be responsible for the occasional excess estimation by the direct method as compared with that by the check method. Apparently the occurrence of this substance in hospital bloods is infrequent. It is possible that Folin's improved phosphotungstic reagent may not react to this substance, so that the new reagent should be used by preference. The color developed by the aid of this reagent is better for estimation than that produced by Folin's older reagent. The latter, however, is fairly satisfactory.

Creatinin estimation—Folin's method¹ was used. Measured portions of blood filtrate and of standard creatinin solution are treated simultaneously with freshly prepared reagent made by mixing picric acid solution with sodium hydroxide and are then compared in the colorimeter.

Sodium chloride estimation—Whitehorn's method² was used. The Cl of blood filtrate is precipitated with standard silver nitrate and the excess of silver remaining in solution is titrated with sulphocyanate using ferric alum as the indicator.

CONCLUSIONS

1. Uric acid determinations cannot be used clinically as a reliable indication of kidney impairment. Uric acid is not retained in the blood at an earlier stage of nephritis than is urea.

2. Creatinin estimation is much more valuable, agreeing approximately with the urea results and serving as a check on the latter. High estimations are very significant for prognosis.

3. Urea estimations are the most reliable and significant of the blood chemistry findings in nephritis. Estimation of urea in blood filtrate by Folin's method using titration instead of desiccation is very advantageous.

4. Sodium chloride estimation yields such variable results that its value in nephritis is very doubtful.

REFERENCES

- ¹Myers, V. C., Fine, M. S., and Lough, W. G. Arch. Int. Med. 1916, LVII, 570.
- ²Baumann, L., Hansmann, G. H., Davis, A. C., and Stevens, F. A. Arch. Int. Med. 1919, XXIV, 70.
- ³Upham, B., and Higley, H. A. Arch. Int. Med. 1919, XXIV, 507.
- ⁴Myers, V. C., and Killian, J. A. Jour. Pharm. and Exper. Therap. 1921, LXVIII, 213.
- ⁵Holbrook, W. P., and Haskins, H. D. JOURN. LAB. AND CLIN. MED., 1926, VI, 377.
- ⁶Lennox, W. G. Jour. Am. Med. Assn. 1924, LXXVII, 602.
- ⁷Harding, V. J., Allen, K. D., Eagles, B. A., and Van Wyck, H. B. Jour. Biol. Chem. 1925, LXXIII, 37.
- ⁸Folin, O. Laboratory of Biological Chemistry, 1922, New York, D. Appleton, p. 230.
- ⁹Benedict, S. R. Jour. Biol. Chem. 1925, LXX, 215.
- ¹⁰Hunter, G., and Eagles, B. A. Jour. Biol. Chem. 1925, LXX, 1.
- ¹¹Folin, O., and Trimble, H. Jour. Biol. Chem. 1924, LX, 417.
- ¹²Folin, O. Laboratory of Biological Chemistry, 1922, New York, D. Appleton, p. 245.
- ¹³Whitehorn, J. C. Jour. Biol. Chem. 1910, 21, 449.

REDUCING SUBSTANCES IN THE URINE*

BY R. D. LEAS, M.D. CLEVELAND, OHIO

THE subject matter in this report is not new material but is presented with the idea of reviewing a few well known but often forgotten facts.

It has many times been recorded that the administration of salicylates will give a reducing substance in the urine, which cannot be differentiated from sugar by the reduction test alone. Other substances giving a similar reaction are uric acid, creatinine, simple aldehydes, formalin and chloroform. Occa-

*From the Department of Medicine of Western Reserve University and The Lakeside Hospital, Cleveland, Ohio.

Received for publication, April 16, 1926.

TABLE I*

CASE NO	DATE	DRUG	AMOUNT OF DRUG	REDUCING SUBSTANCE IN URINE
1	12/27/25	0	0	0
	12/28/25	Sod Sal		0
	12/29/25	" "	540 gr	0 50%
	12/30/25	" "		0
2	12/23/25	Thymol	?	0 316%
	12/24/25	Cinchophen		0 150%
	12/25/25	"		0
	12/26/25	"	500 gr	0 260%
	12/27/25	"		V F T
	12/28/25	"		0 208%
	12/29/25	"		0 277%
	12/30/25	"	0	Less than 0 150%
3	12/17/25	0	0	0
	12/18/25	Cinchophen	210 gr	V F T
	12/19/25	"		F T
	12/20/25		0	0
	12/21/25		0	-
	12/22/25		0	Less than 0 15%
	12/23/25		0	V F T
	12/24/25		0	0
	12/25/25		0	0
	12/26/25		0	0
	12/27/25		0	0
	12/28/25		0	0
	12/29/25		0	Less than 0 15%
	12/30/25		0	V F T
	12/31/25		0	0
4	12/11/25	0	0	0
	12/11/25	Cinchophen	150 gr	0
	12/12/25	"		0 227%
	12/13/25	Sod Sal	640 gr	V F T
	12/14/25	" "		0
5	12/13/25	0	0	0
	12/13/25	Cinchophen		0
	12/14/25	"		0
	12/15/25	"	240 gr	0
	12/16/25	"		V F T
	12/17/25	"		Less than 0 15%
6	12/14/25	0	0	0
	12/14/25	Cinchophen		0
	12/15/25	"		0
	12/16/25	"		0 208%
	12/17/25	"	240 gr	Less than 0 15%
	12/18/25	"		" " 0 15%
	12/19/25	"		0
	12/20/25	"		0 250%
	12/21/25	0		0 316%
	12/22/25	Sod Sal		0 380%
	12/23/25	" "		0 430%
	12/24/25	" "		0 330%
	12/26/25	" "		0 250%
	12/27/25	" "	1760 gr	0 227%
	12/28/25	" "		0 150%
	12/29/25	" "		Less than 0 150%
	12/30/25	" "		" " 0 150%
	12/31/25	" "		0
	1/ 1/26	Tolysin	Gr XV q 2h	0
	1/ 2/26			0
	1/ 3/26			Less than 0 150%

*I wish to thank George Thorngate, MD for his aid in securing the data in this table

TABLE I—CONT'D

CASE NO	DATE	DIUG	AMOUNT OF DIUG	REDUCING SUBSTANCE IN URINE
7	12/ 2/25	0		0
	12/ 2/25	Cinchophen	105 gr	0
	12/ 3/25			V F T
	12/ 4/25	0	0	0
	12/ 5/25	0	0	0
	12/ 6/25	0	0	0
	12/ 7/25	0	0	0
8	3/24/25	0	0	0
	3/25/25	Cinchophen	155 gr	-
	3/26/25			+
	3/27/25	0	0	0
	3/28/25	Cinchophen		0
	3/29/25			0
	3/30/25			0 12%
	3/31/25			+
	4/ 1/25		455 gr	+
	4/ 2/25			0 45%
	4/ 3/25			0 25%
	4/ 4/25			+
	4/ 5/25			0 33%
	4/ 6/25			+
	4/ 7/25			+
9	4/ 2/25	0	0	0
	4/ 2/25	Cinchophen	140 gr	0
	4/ 3/25			0 25%
	4/ 4/25	0		-
	4/ 5/25	0		-
	4/ 6/25	0		-
	4/ 7/25	0		0 25%
	4/ 8/25	0		0
	4/ 9/25	0		0 20%
	4/10/25	0		0
	4/11/25	0		0
	4/12/25	0		0
	4/13/25	Cinchophen	75 gr	0
	4/14/25	Tolysin		+
	4/15/25			-
	4/16/25			0 27%
	4/17/25		Amt undetermined	0
	4/18/25			0
	4/19/25			0
	4/20/25			0
	4/21/25			0
	4/22/25			0
	4/23/25			0 18%
10	5/31/25	0	0	0
	6/ 1/25	Tolysin	825 gr	+
	6/ 2/25			+
	6/ 3/25	0		+
	6/ 4/25	0		+
11	12/ 1/25			
	to	Cinchophen	790 gr	
	12/ 8/25			
	12/ 9/25	0	0	0 547%
	12/10/25	Sod Sal		0 20%
	12/11/25		350 gr	0
	12/12/25			0

TABLE I—CONT'D

CASE NO	DATE	DRUG	AMOUNT OF DRUG	REDUCING SUBSTANCE IN URINE
12	3/14/25	0	0	0
	3/14/25	Cinchophen	75 gr	0
	3/15/25	0		0
	3/16/25	Cinchophen		+
	3/17/25	"		0
	3/18/25	"		+
	3/19/25	"		+
	3/20/25	"	405 gr	+
	3/21/25	"		0 25%
	3/22/25	"		0 18%
	3/23/25	"		0 16%
	3/24/25	"		0 20%
	3/25/25	"		0 31%
	3/26/25	0	0	+
	3/27/25	Cinchophen	30 gr	+
	3/28/25	"	15 gr	-
	3/29/25	0		-
	3/30/25	0		0
	3/31/25	0		0
	4/ 1/25	0		0
	4/ 2/25	0		+
	4/ 3/25	0		0
13	12/ 9/25	0	0	0
	12/10/25	Cinchophen	140 gr	0
	12/11/25	"		0 27%
	12/12/25			0
	12/13/25	Sod Sal		0 15%
	12/14/25	" "		0
	12/15/25	" "		0
	12/16/25	" "	740 gr	V F T
	12/17/25	" "		Less than 0 15%
	12/19/25	" "		0
	12/20/25	" "		Less than 0 15%
	12/21/25			Less than 0 15%
	12/22/25			0
	12/23/25			Less than 0 15%
	12/25/25			Less than 0 15%
	12/26/25			0
	12/27/25			
	12/28/25			
	12/29/25			
	12/30/25			

sionally this leads to incorrect diagnosis, at least temporarily, in a well regulated hospital and may be a more frequent cause of error in the practice of medicine where laboratory methods are not so readily available. In view of the fact that the diabetic patient is not uncommonly afflicted with pains in the legs and elsewhere, and is frequently given salicylates for relief, the occurrence of an additional reducing substance in the urine is very important. These reducing substances are particularly important if a physician is forced to rely on urine sugar as a criterion of the patient's condition.

With these points in mind, urine was collected in twenty-four hour specimens from a series of rheumatic patients before and after they were given sodium salicylate, cinchophen and tolysin. The urine was tested with Benedict's qualitative and quantitative reagents for reducing substances. In all cases fermentation, phenyl hydrazin and polariscopic tests were also made to prove the absence of sugar. In a few instances sugar tolerance tests were also made during the time the reducing substance was present in the urine.

The following tables show that patients receiving cinchophen, sodium salicylate or tolysin invariably have reducing substances in the urine which do not ferment with yeast, give no osazone formation, and rotate the plane of polarized light to the left. The time of their appearance in and disappearance from the urine depends upon dosage and also individual factors which are variable.

The above mentioned drugs themselves with the exception of tolysin do not reduce Benedict's solution *in vitro*, and none gives a positive fermentation test. Another interesting observation was the constant disappearance of these reducing substances from the urine after varying periods of time (twenty four to eighty six hours).

Fermentation and phenyl hydrazin tests on the urine when the reducing substance was present gave a positive reaction in no instance. The urine invariably rotated the plane of polarized light to the left.

TABLE II

CASE NO	DATE OF URINE SPECIMEN	REDUCING SUBSTANCE	REDUCING SUBSTANCE IN 24 HR	REDUCING SUBSTANCE IN 48 HR	REDUCING SUBSTANCE IN 72 HR	REDUCING SUBSTANCE IN 96 HR
1	12/-9/2	+	0			
2	12/27/25	+	0			
3	12/28/25	+	0			
6	12/22/25	+	+		1 F T	0
6	12/27/-	+	+	0		
13	12/27/25	+	0			
Case not reported in Table I		12/20/25	+	+	1 F T	0

Sugar tolerance tests were done on cases 8, 9, and 12 on April 7, January 7, and March 28 with the following results:

CASE 8

Blood sugar	0 11%
Blood sugar 45 minutes after 50 gm glucose by mouth	0 15%
Blood sugar 1 1/4 hours after 50 gm glucose by mouth	0 11%

CASE 9

Blood sugar	0 10%
Blood sugar 45 minutes after 50 gm glucose by mouth	0 15%
Blood sugar 1 1/4 hours after 50 gm glucose by mouth	0 15%

CASE 12

Blood sugar	0 10%
Blood sugar 1 hour after 50 gm glucose by mouth	0 15%
Blood sugar 1 1/4 hours after 50 gm glucose by mouth	0 10%

SUMMARY

- 1 The purpose of this paper is to recall the fact that cinchophen, salicylates and tolysin give reducing substances in the urine which may be confused with sugar by the copper reduction test alone.
- 2 Data are given on thirteen cases.
- 3 These reducing substances do not ferment nor produce osazones but rotate the plane of polarized light to the left.
- 4 Their presence and quantity in the urine does not depend upon dosage alone.
- 5 They disappear from specimens of urine in from twenty four to eighty six hours.

PROPHYLACTIC AND THERAPEUTIC POSSIBILITIES OF THE
TWORT-D'HERELLE'S BACTERIOPHAGE
(PRELIMINARY PAPER)

BY LLOYD ARNOLD, M D , AND EMIL WEISS, M D , CHICAGO, ILL

D'HERELLE has applied the term bacteriophage to the phenomenon which consists essentially in a dissolution or lysis of bacteria through the operation of a principle which he has called bacteriophage. This lytic substance has been used as a prophylactic and a therapeutic agent in some infectious diseases¹. The usual method of obtaining this bacteriophage, that is to be used for such purposes, is by adding a small amount of the lytic agent to a broth suspension of a young culture of the susceptible bacteria and incubating until clearing of the broth takes place. This cleared or lysed culture is then passed through a Berkefeld candle, and the sterile filtrate is used for prophylactic and therapeutic purposes. During the process of lysis of the young bacteria the active lytic principle is increased in concentration.

We have used another method of preparation of the bacteriophage in this laboratory². A layer of sterile 2 per cent agar in distilled water is poured into a Petri dish, this is covered with a piece of sterile tissue paper, and a layer of nutrient agar is poured on top of this paper. A drop of the lytic agent and a loop of susceptible bacteria are added to the surface of this top layer in the usual manner and smeared well with a sterile bent-glass spreader. After twenty-four hours incubation, the top layer with the irregularly shaped, so-called lysogenic colonies is removed with the adjacent tissue paper. This layer is discarded. The bottom layer is now removed and extracted with distilled water and the extract passed through a Berkefeld filter. Experiments have shown that this layer contains a strong bacteriophage and a minimum content of bacterial proteins³. For convenience of description, the broth cleared growths will be referred to as d'Herelle's method and the latter procedure as the bottom-layer extraction method.

It is apparent that either of these filtrates contains dissolved bacterial proteins and the by-products of metabolism of the bacteria that have grown in the culture medium. The culture of bacteria must be actively growing before lysis takes place. We have no evidence of lytic activity taking place in adult bacteria. Lysis reaches its maximum during the period of the life cycle of the bacteria when they are reproducing by geometrical progression. It is interesting in this connection to recall that Levaditi⁴ has observed that the vaccinia virus is only proliferated in actively growing ectoendodermic cellular elements, he thinks that there must be a "karyokinetic rejuvenescence" as a result of a previous irritation before the virus can be cultivated in such tis-

¹Department of Bacteriology Pathology and Preventive Medicine Loyola University
School of Medicine Chicago
Received for publication February 5 1926

the The toxins in cultures of *B. tetani*, *B. diphtheria*, and such 'exotoxin' producing bacteria do not reach their maximum concentration until after several days incubation, in fact, there are several days intervening between the period of growth and that of the highest toxin titer. This is also true for *Staphylococcus aureus* toxin.⁵ The hemolysins produced by some strains of staphylococci are not formed until several days after maximum growth has occurred.⁶ The hemolysins produced by streptococci seem to reach their highest titer at the time of maximum growth although these lysins seem to be nonantigenic.^{7, 8}

In the use of bacteriophage for prophylactic and therapeutic purposes it is necessary to distinguish between the effects produced by the bacterial proteins and those produced by the active lytic agent. It is logical to suppose that if only lysis occurred the dissolved bacterial proteins would constitute an ideal antigen. When vaccines are injected for immunization purposes we think the dead bacteria are dissolved, lysed, or broken up by the ferments that are in the fluids and cells present in the area during the stage of inflammation caused by the presence of the injected bacteria. The bacterial protein liberated in this manner is carried away by the blood and lymph stream and serves as the antigen that causes the production of antibodies. Then if we possessed a lytic agent capable of producing lysis but not accompanied by proteolysis, we should have an ideal antigen.

Several workers⁹ have attempted to show that the lysis that occurred as the result of phage activity was not accompanied by proteolysis. These results are not conclusive, they only show that probably proteolysis does not take place. All observers have noticed that a broth inoculated with a susceptible bacteria and phage grows very rapidly for the first part of the logarithmic phase of its life cycle before lysis begins.¹⁰ Bacterial counts often show the total number of bacteria increased beyond that number present in the control tube without phage at the time just previous to rapid lysis or clearing of the visible bacterial suspension. We have many reasons to think that during the active lytic period there is continuous growth and prompt lysis etc. These two phenomena are most probably going on at the same time. The rapid clearing of the culture as well as the development of secondary visible and phage resistant strains in the culture probably depend upon these two processes that are going on at the same time. Arnold and Weiss³ used the total nonprotein nitrogen, and since then we have used the Van Slyke method of determining amino nitrogen. Ionescu Mihailescu⁹ used formal titration. Both workers were unable to demonstrate a marked difference between the lysed culture and the control culture of the same age. We do not know as yet how it is possible to have a proper control that will represent the same number of bacteria that are represented in the unknown lysed culture.

Douglas¹¹ has shown that short periods (four to twelve hours) of tryptic digestion of acetone extracted Shiga vaccine causes a change in its antigenic properties. The agglutinating and opsonic antibodies do not appear in the immunized animals' serum. The bactericidal, precipitating and neutralizing powers are developed as in the control vaccine treated animals. Allison¹²

using "lysozyme" from egg white to dissolve *Streptococcus fecalis*, found the bacteriolysins and agglutinins absent, the opsonins diminished, but the bactericidal power and the complement-fixing antibodies were the same as in the vaccine-injected controls. We do not wish to discuss the question of the unity of antibodies, but we have shown in former work^{3, 13} that there were present in the serum of animals injected with phage lysed cultures all of the antibodies that could be demonstrated in the control animals injected with dead (vaccine) and living bacteria. This seems to us to be more conclusive evidence than the uncontrollable chemical results already mentioned that proteolysis does not accompany bacteriolysis due to phagic action.

The bacteria dissolved by phage are young growing bacteria. Their protein content should make good antigenic material. Heat, attenuation, and chemical changes due to antiseptics, etc., are avoided. We can conclude then that probably the soluble bacterial proteins in the sterile filtrate of the lysed culture of bacteria are ideal antigens theoretically.

All the studies that have been made with the use of bacteriophage as a prophylactic and a therapeutic agent have had in the injected material these lysed bacterial proteins as well as the transmissible bacteriolytic principle. We have recently published a method of obtaining a bacterial protein-free bacteriophage.¹⁴ Since this work has been published, we have attempted to purify further the bacteriophage, but up to the present we have not succeeded in doing so. We will refer to the usual bacteriophage as "bacterial protein phage" and to the purified bacteriophage as "bacterial protein-free phage."

Bruynoghe and Maisin¹⁵ and Gratia¹⁶ injected d'Herelle's bacteriophage subcutaneously in normal individuals. They describe a febrile reaction with chills, insomnia, etc., persisting for about forty-eight hours. Locally the injections produced erythema and edema which lasted for several days. The reaction was the same if antityphoid or antistaphylococcus bacteriophage were used. The reaction was, in other words, what one would expect to follow any injection of a material containing the amount of foreign proteins present in the bacteriophage they used.

Coucoux, Philibert, and Cordey,¹⁷ Munter and Boenheim,¹⁸ and Zdansky¹⁹ have treated infections of the urinary tract by subcutaneous injections of bacteriophage. Gratia²⁰ and Gougerat and Peyre²¹ have treated staphylococcus skin infections by subcutaneous injections of a staphylococcus-bacteriophage. McKinley²² treated several different infections with subcutaneous injections of bacteriophage. Beckerich and Handuroy,²³ and d'Herelle¹ have used the subcutaneous injections of bacteriophage in the treatment of typhoid fever. This is by no means a literature review of the various infections treated with subcutaneous injections of bacteriophage but illustrates the variety of diseases that have been so treated by some workers. In reading these reports, the nonspecific or foreign protein reaction is outstanding where a clinical improvement has been reported. The injection of the bacterial protein-free bacteriophage does not cause a change in the distribution of the peripheral leucocytes or a change in the heat regulative mechanism of rabbits. The antibacteriophage content of the serum of animals after bacterial protein-

free bacteriophage has been injected is very high, but there is no evidence of antibacterial bodies in such serum. We wished to study experimentally in the rabbit the relative protective action of bacteriophage, as we are now preparing it, free from antigenic bacterial proteins.

EXPERIMENTS

Time of Appearance of Bacterial Antibodies After Injection of Dead, Living, and Phage Lysed B Typhosus

Rabbits were immunized by intravenous injections of bacterial protein bacteriophage (typhoid), B typhosus vaccine and living B typhosus. The vaccine was prepared by heating a twenty four hour old broth culture to 60° C for one hour. The living bacteria used were twenty four hour old broth cultures. The first injection was 0.5 cc of each after seven days 1 cc, after fourteen days 2 cc and after twenty one days 3 cc. On the seventh, fourteenth, and twenty first days just before reinjection, blood was taken to determine the agglutinin and opsonic titer against B typhosus. Table I gives in condensed form the results of this experiment. The bacteriophage containing the filtered, lysed B typhosus stimulated or caused to appear in the blood agglutinins and opsonins quicker than did the dead and living B typhosus. The ultimate titer was never so high as was found with the use of the latter two as antigens. This experiment has been repeated using 24 rabbits instead of the 12 recorded in Table I. The results were the same.

TABLE I

AGGLUTININS AND OPSONINS PRODUCED BY TYPHOID BACTERIOPHAGE LIVING AND DEAD TYPHOID BACILLI

ANTI BODIES	ANTIBODIES OF												
	NO OF RAB-BITS	TYPHOID BACTERIOPHAGE				LIVING TYPHOID BACILLI				DEAD TYPHOID BACILLI			
		DATE OF TITRATION											
		10/10	10/17	10/24	10/31	10/10	10/17	10/24	10/31	10/10	10/17	10/24	10/31
Agglutination Titer	1	160	1000	2000	4000	20	10000	64000	128000	10	2000	16000	16000
	2	20	1000	3000	4000	10	8000	32000	20000	10	4000	32000	64000
	3	160	1000	6000	8000	20	4000	64000	128000	10	4000	16000	32000
	4	80	1200	5000	6000	40	6000	64000	128000	20	8000	32000	32000
Opsonic Index	1	6.4	9.1	16.3	24.5	4.1	19.5	43.1	70.2	3.3	14.1	26.7	27.6
	2	6.9	8.5	15.1	22.4	3.6	17.6	31.5	33.1	2.8	17.0	31.6	45.3
	3	6.2	9.3	14.1	21.7	2.8	15.8	38.7	62.6	3.9	20.1	24.8	51.5
	4	5.9	8.4	13.6	19.2	4.5	16.6	45.9	81.4	3.6	19.2	32.7	34.8

All animals were injected upon 10/3 10/10 10/17 and 10/4

We are convinced that these results cannot be attributed to individual variation in the rabbits used by us. The same experiment was carried out with the use of B dysenteriae Shiga as the antigen. On account of the toxicity of Shiga for rabbits 0.25 cc was injected intravenously as the initial dose. Shiga phage, vaccine and living bacteria were used. One set of rabbits were immunized with the phage at two day intervals instead of seven day intervals. This was done to see if the interval selected had an influence upon the antibody response of the organism against the lysed bacterial proteins. Twenty

TABLE II
AGGLUTININS AND OPSONINS PRODUCED BY INJECTION OF SHIGA BACTERIOPHAGE, LIVING, DEAD SHIGA BACILLI, AND SHIGA AUTOLYSATES*

ANTI BODIES	NO OF RAB BITS	SHIGA BACTERIOPHAGE SEVEN DAY INTERVALS				SHIGA BACTERIOPHAGE TWO DAY INTERVALS				LIVING SHIGA BACILLI				DEAD SHIGA BACILLI				SHIGA AUTOLYSATES			
		10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6
Aggluti- nation Titer	1	1500	3000	4000	8000	650	2300	7000	14000	400	10000	25000	50000	200	5000	14000	30000	100	2300	10/2, 10/11	11/6
	2	1000	2500	1500	9000	1000	3000	8500	16000	250	8000	20000	33000	100	3000	9000	23000	80	4000	8700	20000
	3	600	1800	3200	6500	800	2500	6000	12000	300	9000	30000	73000	350	6000	16000	40000	40	2000	9000	18000
Opsonic Index	1	1200	2000	4000	8500	600	2000	4500	10000	400	8000	26000	40000	150	4500	12000	36000	160	3000	10000	25000
	2	121	138	162	185	84	142	174	256	49	167	266	486	32	128	227	380	26	131	198	291
	3	85	123	151	166	93	136	187	261	38	152	229	316	29	119	182	353	21	158	182	257
	3	73	142	175	188	96	157	168	224	31	175	285	598	36	162	235	413	18	126	191	242
	4	89	121	159	175	71	119	155	202	41	113	273	422	31	121	211	395	33	133	203	316

*First injection was made on 10/9 others followed at intervals noted in table

four hour old agar slants of Shiga were each washed off with 5 c c of distilled water, placed at room temperature for four days, and passed through a Berkefeld candle. This filtered Shiga autolysate was used as an antigen. The results of this experiment are given in Table II. The bacterial protein phage causes the appearance of antihodies sooner than living, dead, or autolysed Shiga bacilli. The ultimate titer is not so high after phage injection. This experiment was repeated again, using another 20 rabbits. The results did not differ from those recorded in Table II.

Relative Degree of Protection Against Lethal Dose of Homologous Bacteria after Injection of Dead, Living, and Phage Lysed B Dysenteriae Shiga

We were interested in the question as to whether a rabbit would be protected against a lethal dose of Shiga bacilli after immunization with the bacterial protein phage to the same extent as would be shown after vaccine, living, or autolysed Shiga. Sixteen rabbits were injected as in the previous experiment—4 with Shiga phage, 4 with dead, 4 with living, and 4 with autolysed Shiga bacilli. The initial intravenous injection was the same. After seven days blood was taken to test for agglutinins, and 1 rabbit of each series with 2 uninoculated controls was given intraperitoneal lethal dose of Shiga bacilli (one half of a twenty four hours old agar slant culture). The only rabbit that survived was the 1 that had received the 0.25 c c Shiga phage seven days previously. All the other 3 with the 2 controls died between forty eight and seventy two hours. The remaining 3 rabbits of each series were reinjected on the seventh day as in the Shiga agglutinin experiment. Seven days after the second injection, 1 rabbit of each series was given $1\frac{1}{2}$ lethal doses of Shiga bacilli. The only one to die was the autolysate rabbit. The other 3 were not killed by the $1\frac{1}{2}$ lethal dose. Seven days after the third injection, 1 rabbit of each series received 2 lethal doses of Shiga bacilli. All survived. Seven days after the fourth injection the remaining rabbit of each series was given 3 lethal doses of Shiga bacilli. All survived. The intravenous injection of the phage with its lysed bacterial protein content, protects the rabbit against a lethal dose of the homologous bacteria sooner than does the living, or dead bacteria, or their autolysates. Topley⁴ records a protection in mice against a lethal dose of *B. aertrycke* when the phage lysed filtrate is injected intraperitoneally fourteen days before the lethal dose of the homologous bacteria is given.

D'Herelle¹ claims that the larger the dose of the bacteriophage the greater the delay in the immunity. This seems unreasonable, unless the dose is so large that the bacterial protein content is sufficient to cause intoxication and interfere with antibody production. He does not mention this latter reaction as having occurred in the barbours in buffaloes which he used for this work. We injected 3 rabbits with 0.2 c c bacterial protein Shiga phage, 3 with 1 c c, 3 with 2 c c, and 3 with 4 c c of the phage. After seven days 1 from each set was given 3 lethal doses of the living Shiga bacilli. All lived and did not show any signs of a reaction. After fourteen days 1 rabbit from each series was given 6 lethal doses and after twenty one days the remaining rabbit in each series received 12 lethal doses. All of the animals lived in both experi-

ments The larger dose did not delay the protection within the period of our experiment

D'Herelle observed hypersusceptibility to infection of the homologous bacteria when animals were repeatedly injected with bacteriophage We have never observed this reaction, although over 60 rabbits have been injected with our bacterial protein-free bacteriophages, typhoid, Shiga, and staphylococcus Rabbits that have been injected with purified bacteriophage contain in the serum a high titer of antibacteriophagic bodies, but they are just as susceptible to the lethal dose of the homologous strain as are the normal uninjected control animals Where there is a contamination of the phage with the homologous bacterial proteins, protection has always been observed against the lethal dose of the same bacteria

The Toxicity of Bacteriophage

D'Herelle states that old bacteriophage is not as toxic as freshly prepared phage We have substantiated this observation Five cubic centimeters of a freshly prepared typhoid bacteriophage is the lethal dose for a rabbit The same amount of old phage makes the animal sick for a day, but it always recovers We have treated freshly prepared and ten months old typhoid bacteriophage with nine parts of 15.55 per cent sodium sulphate solution for two hours at 37° C The precipitate was collected on filter paper and redissolved in an amount of normal salt solution equal to the original volume of bacteriophage This solution was then passed through a Berkefeld candle Five cubic centimeters of this redissolved precipitate, containing the bacterial proteins fraction of the freshly prepared typhoid phage, is as toxic as the same amount of the original material Five cubic centimeters of the redissolved precipitate of the ten months old phage has the same toxicity as a corresponding amount of old bacteriophage We conclude that the toxicity manifested upon injection of bacteriophage is due to the bacterial protein and other precipitable material

It is well known that rabbits are more susceptible to *B. dysenteriae* Shiga than they are to *B. typhosus* We have found that the Shiga phage is not so toxic as typhoid phage Five cubic centimeters of Shiga phage caused a slight reaction, but after the second day the animals returned to normal Probably there is only a small amount of exotoxins present in such lysed filtrates, while the bacterial protein and endotoxin content is very high

Experimental Therapeutic Use of Bacterial Protein-Containing and Bacterial Protein Free Bacteriophage

We have described in detail our method of the quantitative estimation of bacteriophage¹³ This method consisted, briefly, of determining the smallest amount of bacteriophage added to the surface of an agar plate, seeded with a standard loop of susceptible bacteria, that would cause all the colonies to be irregular or lysogenic in outline All observers have noted that a large dose of phage would cause inhibition of growth of susceptible bacteria In this experiment we have titrated the amount of bacteriophage that would inhibit the growth of a given dose of bacteria After the lethal dose of the

with relatively few colonies of the lysogenic type, *F'* was the same as *B* plate Within the limits of our experiment, the in vivo lysogenic activity of the bacteriophage and susceptible bacteria were roughly proportioned to the in vitro inhibitory activity

TABLE III
PHAGOCYTIC INDEX AFTER INTRAVENOUS INJECTION OF B DYSENTERIAE SHIGA AND BACTERIOPHAGE
(Experiment II)

NO OF FAB BITS	MATERIAL INJECTED	WHITE BLOOD COUNT AFTER INJECTION						PHAGOCYTIC INDEX AFTER INJECTION					
		BEFORE	1	3	6	12	24	1	3	6	12	24	
		HOURS						HOURS					
1	1 Lethal dose of Bacilli Shiga	6700	16300	5900	6100	6700	6200	0.9	0.82	0.9	0.75	0.88	
2	1 Letha Dose of Bacilli Shiga	7150	19250	7100	7600	8150	8400	0.76	0.6	0.8	0.9	0.7	
3	1 Lethal Dose Corresponding Amount of + Rou tino Phage	8200	15100	10200	8900	9100	7800	1.6	1.1	0.5	0.65	0.71	
4	1 Letha Dose of Bacilli Shiga	9200	18700	9450	8300	6900	7500	1.3	1.05	0.9	0.91	0.84	
5	1 Lethal Dose + 3/4	8300	16900	8100	9100	7600	8200	1.02	0.95	0.89	0.92	0.85	
6	1 Letha Dose of Bacilli Shiga	8700	19600	7650	8350	6700	9400	1.15	0.98	0.9	0.86	0.78	
7	1 Lethal Dose + 1/2	8100	14500	10200	9300	9100	8600	1.08	0.91	0.83	0.76	0.82	
8	1 Letha Dose of Bacilli Shiga	7300	15200	9800	8100	8800	7700	1.03	0.99	0.94	0.8	0.91	
9	1 Lethal Dose + 1/4	6200	13100	7500	7100	8500	8100	1.09	1.01	0.86	0.86	0.74	
10	1 Lethal Dose of Bacilli Shiga	7850	16300	9200	8650	8900	8700	1.05	0.94	0.92	0.85	0.71	

EXPERIMENT III

- A Lethal dose of B dysenteriae Shiga -----died 3-4 days
- B Lethal dose of B dysenteriae Shiga and inhibiting dose of bacterial protein phage, given immediately -----slight reaction, living
- C Lethal dose of B dysenteriae Shiga and inhibiting dose of bacterial protein phage, given after twenty four hours-----reaction lasting 4-7 days, living
- D Lethal dose of B dysenteriae Shiga and inhibiting dose of bacterial protein phage, given after three days-----One died immediately after phage injection, one on the fourth and one on the fifth day after Shiga injection

Bacteriolysis in vitro cannot be compared with the same phenomenon in vivo There is no conclusive evidence that there is bacteriophage lysis in vivo These and other similar experiments show that the lytic agent must be administered in sufficient quantity to exercise a growth inhibitory effect upon the bacteria The phagocytic index was determined from the blood from the same rabbits The results are recorded in Table III We have substantiated d'Herelle's observation that his bacteriophage causes an increase in the phagocytic power of leucocytes ^{1 3} We did this experiment to find out what part phagocytosis might play in the above reaction We think we are justified in concluding that phagocytosis does not play an important rôle in the reaction, certainly it only plays a secondary rôle

We did Experiment III to determine the therapeutic value of the bacteriophage administered at different intervals after the injection of the lethal

dose of the Shiga bacilli. Three rabbits were used for each series, 12 rabbits in all. We have repeated this experiment on 12 more rabbits varying the time of phage injection from eighteen to thirty hours after the lethal dose of Shiga bacilli. In most instances after the onset of paralysis of the extremities, the phage injection leads to recovery. As has been found with antitoxins, when retrogressive changes in certain tissues are extensive the restoration to the normal does not take place. The experimental therapeutic administration of phage in Shiga infection in the rabbit substantiates d'Herelle's observation. We wish to mention that Shiga phage is one of the most nontoxic bacteriophages we have worked with. It does not compare in toxicity with typhoid phage. For experimental therapeutic use of this phage we have had to free it of bacterial proteins and other toxic substances.

DISCUSSION

All of the antibodies that can be demonstrated in the blood of an immunized animal after the use of *B. typhosus*, living, dead, and autolyzed can be found after immunization with phage lysed filtrates of *B. typhosus*. In addition, we find the antibody titer develops more rapidly and the protection against a lethal dose of the homologous bacteria is manifested more quickly after a single injection of the phage lysed *B. typhosus* than after a similar injection of living vaccine or autolysates of the same bacteria although the ultimate antibody titer is not so high. All available evidence seems to indicate that phagic bacteriolysis is not accompanied with proteolysis.

The degree of protection against lethal doses of the homologous bacteria as a result of a single injection of phage lysed *B. typhosus*, *B. dysenteriae*, Shiga, and *Staphylococcus aureus* is out of proportion to the demonstrable antibody titer in the blood serum at the time that this immunity is enjoyed by the animal.

When phage lysed filtrates are used for curative or specific therapeutic treatment, the toxicity of the particular protein must be taken into consideration. If the injection of the bacterial protein is accompanied by evidences of intoxication, these proteins should be removed by some of the methods described by us in a former publication.¹³ Our results so far indicate that the beneficial effects obtained by phage injections following the administration of lethal doses of the homologous bacteria in rabbits are due to the growth inhibitory effect of the bacteriophage. If smaller doses of phage are administered, these do not protect the animal against the lethal effect of the bacterial infection. In the test tube much smaller doses than these lead to bacteriolysis with ultimate depression of the bacterial growth. This is due to lysis that occurs during the period of maximum growth *in vitro*. Large doses of bacteriophage *in vitro* lead to inhibition of growth. *In vivo* we have found that this dose was necessary to save the animal after administration of a lethal dose of the homologous bacteria. We have been led to believe that the bacteriophage does not increase in *in vivo* as it does in *in vitro* experiments. At least this has not been observed under the conditions of our experiment.

The transmissible bacterial lysins (bacteriophage), freed from toxic lysed

bacterial proteins and administered in sufficient quantity, may offer a curative or therapeutic aid in certain infectious diseases

In considering bacteriophage from a clinical standpoint, one must separate the bacterial proteins from the active transmissible lytic agent (phage) In all of the work so far recorded, both factors were considered together Since we have succeeded in separating these two components, we can now determine their respective therapeutic and preventive properties From our experimental work we think the bacterial protein content of the bacteriophage used heretofore has been the active agent in the improvement in the cases so far recorded It would be impossible to inject a dose of bacteriophage large enough to cause inhibition of *B typhosus* in vivo in typhoid fever because of the toxicity of the accompanying bacterial proteins

We have not mentioned the administration of bacteriophage per os There is an entirely different mechanism involved in this reaction Considerable more work must be done upon the bacteriology and physiology of the upper end of the gastro-intestinal tract²⁵ before we can hope to utilize per os administration of bacteriophage intelligently

SUMMARY

The lysed, soluble bacterial proteins in the bacteriophage are antigenic and confer an early active immunity

Bacteriophage, free of antigenic bacterial proteins, prevents death when injected in the rabbit after a lethal dose of bacteria has been given

REFERENCES

- 1d'Herelle Immunity in Natural Infectious Diseases, Baltimore, 1924, Williams and Wilkins
- 2Arnold, L Bacteriophage Phenomena, *Jour of Lab and Clin Med*, 1922 23, viii, 720
- 3Weiss, E, and Arnold, L A Study of Antigenic Properties of Bacteriophago, *Jour Infect Dis*, March, 1924, xxxiv, 317
- 4Levaditi, C Researches on Vaccine Virus, *Jour State Med*, April, 1924, xxxii, 151
- 5Parker, J T Production of an Exotoxin by Certain Strains of *Staphylococcus Aureus*, *Jour Exper Med*, 1924, vi, 761
- 6Julianelle, L A Studies of Hemolytic *Staphylococci*, *Jour Infect Dis*, 1922, xxxi, 256
- 7McLeod, J W On the Hemolysis Produced by Pathogenic *Streptococci* and in the Existence of Anti Hemolysin in the Sera of Normal and Immunized Animals, *Jour Path and Bacteriol*, 1912, xvi, 321
- 8Stevens, F H, Brady, J W S, West, R Relation between the Virulence of *Streptococci* and Hemolysin, *Jour Exper Med*, 1921, xxxiii, 223
- 9Besredka Ann de l'Inst Pasteur, 1901, xi, 880
- 10Ionescu Mihailesti, C Studies of the Twort d'Herelle Phenomenon, *Jour Exper Med*, 1924, xl, 317
- 11Doerr, R Die Bakteriophagen Phenomen Von Twort und d'Herelle *Klin Wchnschr*, I No 2, p 1489, 1922
- 12Douglas, S R On Some Characters of the Cleavage Products of Certain Bacteria, etc, *Brit Jour Exper Path*, 1921, ii, 175
- 13Allison, V D Antigenic Properties of Lysozyme dissolved Vaccines, *Brit Jour Exper Path*, 1925, vi, 99
- 14Arnold, L, and Weiss, E A Study of Bacteriophago with Antibacteriophage Serum, *Jour Infect Dis*, November 1924, xxxv, 505
- 15The Twort d'Herelle Phenomenon, The Resemblance of Bacteriophage to Toxins and Ferments, *Jour Infect Dis*, December, 1924, xxxv, 603
- 16Arnold, L, and Weiss E *Jour Infect Dis*, 1925, xxxvii, 411
- 17Bruynoghe, R, and Maisin, J Au Sujet de la Reaction consecutive a l'Injection du Bacteriophage, *Compt rend Soc de biol*, 1922, lxxvii, 294
- 18Gratia, A, and Jaumain, D Au Sujet des Reaction consecutive aux Injections de Principe lytique staphylococcique, *Compt rend Soc de biol*, 1922, lxxvii, 519

- ¹¹Courcoux, Philibert and Cordey Un Cas de pyélonéphrite gravidique traitée par le Bactériophage de d'Herelle, Bull et mem Soc Méd d hop de Paris 1922, xlv Series 3 p 1151
- ¹²Münter H and Boenheim, C Ueber therapeutische Versuche mit Bacteriophagen Lysin bei Kindern und Säuglingen Ztschr f Kinderh Mai 22 1925 xxxiv 348
- ¹³Zdarsky, E Ueber die Bakteriophagie und die Möglichkeit ihrer therapeutischen Verwendung Seuchenke Kemptung, 1923, ii 150
- ¹⁴Gratin, A La Lyse transmissible du Staphylocoque Sa Production ses Applications therapeutiques, Compt rend Soc de biol 1922 lxxvii 276
- ¹⁵Gougerot and Peyre Le Bacteriophage dans le Traitement des Affections cutanées Compt rend Soc de biol 1924 cxi 432
- ¹⁶McKinley E B The Bacteriophage in the Treatment of Infections Arch Int Med 1923 xxvii, 899
- ¹⁷Beckerich, A and Handurov, P Le Bacteriophage dans les Traitement de la Fièvre typhoid, Compt rend Soc de biol 1922 lxxvii 118
- ¹⁸Topley, Wilson, and Lewis The Role of the Tward Herelle Phenomenon in Epidemics of Mouse Typhoid, Jour Hyg 1923 xxvii 17
- ¹⁹Arnold, L, and Brody, L Bacterial Flora and Hydrogen ion Concentration of the Duodenum Jour Infect Dis 1926 xxxviii 249

THE ELECTIVE LOCALIZATION OF BACTERIA IN HEART AND VASCULAR DISEASE*

By RUSSELL L. HADEN, M.D. KANSAS CITY, MO.

THE reproduction of a patient's lesion in animals by the injection of bacteria recovered from a septic focus is most valuable proof of a causal relation of the focus to the systemic disease. Such proof depends upon the fact that bacteria in foci of chronic infection may acquire certain properties which determine their localization on introduction into the experimental animal. This fact was first pointed out and has been repeatedly emphasized by Rosenow.¹ I have studied the elective affinity of bacteria isolated from chronic foci in patients suffering from metastatic infections of the eye and kidney infections,² and peptic ulcer,³ and reported experimental results confirmatory of Rosenow's theory. Recently I have been interested in determining the results of the inoculation of bacteria, principally streptococci, from a series of patients suffering from metastatic heart and vascular disease.

It has long been recognized that endocarditis is of bacterial origin, although a great variety of organisms may give rise to vegetations on the valves. Likewise it is now known that disease of the myocardium is largely the result of the activity of bacteria or their toxins. Numerous investigators have produced endocarditis experimentally. Rosenow⁴ has studied the problem of the specific affinity of certain organisms for the heart valves. He found that 84 per cent of the rabbits inoculated with streptococci isolated from foci in patients suffering from lesions of the heart valves had endocarditis at autopsy. Only 14 per cent of the rabbits injected with strains from other sources had similar lesions. Detwiler and Robinson⁵ found also that streptococci recovered by blood culture in patients with active endocarditis involved the valves of a high percentage of the rabbits injected. Thalhimer and Rothschild⁷ studied the myocardial lesions found in rabbits

*From the Department of Medical Research, Deanner Institute, Kansas City, Missouri.
Received for publication March 15, 1926.

inoculated with streptococci from various sources. In most cases no gross lesions were found. Henric^s produced both valvular and myocardial lesions in rabbits with different types of streptococci.

METHOD

All inoculations in my series have been made with organisms, usually streptococci, from dental infection. The method of obtaining the culture material, making the culture, and other technical details have been described fully elsewhere.² Rabbits have been injected intravenously with the original broth cultures. The animals averaged about 1500 gm. Each animal was given 5 cc of the broth suspension. The animals were killed in three to five days after injection and all organs carefully examined for lesions. No lesion was considered present unless plainly visible to the naked eye of at least two observers.

EXPERIMENTAL OBSERVATIONS

Forty rabbits were inoculated intravenously with the cultures from 10 patients suffering with heart or vascular disease of bacterial origin. At least 2 rabbits were injected from each patient. Eighty-two per cent of animals injected had, at autopsy, some gross heart lesion, 63 per cent showed involvement of the endocardium, 50 per cent had gross myocardial disease (Table I). During the period covered by these experiments 1210 other rabbits were injected with cultures from dental foci in patients not known to be suffering from heart or vascular disease. Of these only 17 per cent had vegetations or hemorrhage of the endocardium, only 9 per cent showed myocardial involvement. Table I shows also the percentage of animals having lesions in other organs. The figures other than those for the heart are much the same in the 2 groups.

TABLE I

LOCALIZATION OF BACTERIA FROM INFECTED TEETH IN HEART AND VASCULAR DISEASE

GROUP	NUMBER OF ANIMALS	NUMBER OF PATIENTS	PERCENTAGE OF ANIMALS SHOWING LESIONS IN							STOMACH AND DUODENUM
			JOINT	KIDNEY	MUSCLE	ENDO CARDIUM	MYO CARDIUM	BRAIN	EYE	
I*	1210	405	60	32	22	17	9	5	14	14
II†	40	10	60	25	22	63	50	2	8	14

*Group I—Animals inoculated with dental cultures from patients not known to be suffering from heart or vascular disease.

†Group II—Animals inoculated with cultures from teeth of patients suffering from heart or vascular disease.

The valvular lesions were almost entirely vegetations, often of large size. Occasionally they were large enough to almost completely occlude the valve opening. In some animals only hemorrhages at the base of the valves were found. In 24 animals a record was kept of the valves involved, 17 animals had involvement of the tricuspid valve, 7, the mitral valve. The myocardial involvement consisted often of gross hemorrhage in the heart muscle. In about an equal number of cases the lesion consisted of focal necrosis, usually multiple. These appeared as short, white streaks best seen

when fresh. They are similar to the focal lesions seen in the voluntary muscles.

The case histories and protocols of the animal experiments are given in detail later.

SUMMARY AND CONCLUSIONS

Forty rabbits were inoculated intravenously with bacteria from the infected teeth of a few patients suffering from heart or vascular disease. Eighty-two per cent of the animals showed some heart lesion, 63 per cent had valvular disease, and 50 per cent showed myocardial involvement.

During the same period 1210 rabbits were injected similarly with cultures from patients not known to have heart or vascular disease. Twenty-two per cent of these had some heart involvement, 17 per cent had valvular lesions, and 9 per cent, myocardial disease.

These results are confirmatory proof of Rosenow's theory of elective localization. They emphasize also the possible relation of the dental infection to the heart disease.

CASE REPORTS

Acute Myocarditis

CASE 1 History—D. H., a medical student aged twenty-four years, stated that he had had several acute attacks of rapid heart beginning at the age of twelve years. There was no history of coincident infection at the onset. At the age of eighteen an attack occurred with an abscessed tooth. In May 1925 he had an attack lasting several hours during which electrocardiograms were taken. These showed the tachycardia to be of a ventricular type (Fig 2 A B C). For several weeks before this attack he had had an infection around a partially erupted third molar tooth. A second electrocardiogram taken in June 1925 showed a normal heart rate but evidence of myocardial disease (Fig 2 D E F).

Animal inoculations—At this time the third molar tooth was removed, revealing a pocket of pus from which a pure culture of a streptococcus was obtained. Two rabbits were injected with this culture. One died forty-eight hours later. At autopsy there were numerous areas of hemorrhage in the heart muscle (Fig 3 C and D). The other animal was killed. This also had a smaller number of hemorrhages in the heart muscle.

Subsequent course—In October 1925, the patient had another attack during which he died. At autopsy the heart showed no gross lesions. Sections however showed areas of acute infection in the heart muscle (Fig 3 A and B).

Acute Auricular Fibrillation

CASE 2 History—H. B. D., aged sixty-five years, a banker, had been having attacks of acute auricular fibrillation for only a short period of time. He had otherwise been in excellent health. The general physical examination was negative except for the heart condition. He had had some indefinite gastric symptoms. There was no hypertension. The dental radiographs showed 3 pulpless teeth, only 1 of which showed radiographic evidence of infection. All 3 teeth were extracted. Only 2, the upper right and left second bicuspid, were cultured. Both showed a profuse growth of streptococci.

Animal inoculations—Two animals were injected with 5 cc each of the broth culture of the streptococcus recovered from the upper right second bicuspid. One animal was killed five days later. The examination showed a large vegetation on the tricuspid valve (Fig 4 B) and hemorrhages in the myocardium. There were also hemorrhages in the first part of the duodenum, a few cortical kidney abscesses, purulent fluid in the large joints, and some areas of necrosis in the muscle. The second rabbit was killed six days after injection. There were vegetations on the tricuspid valve and in the right auricle (Fig 4 C). The joints showed very slight involvement. There was 1 small abscess in the medulla of 1 kidney and some necrosis in the muscle.

Two rabbits were also injected with the diplococcus recovered from the upper left second bicuspid (Fig 4 A) One showed hemorrhages in the papillary muscle of the left ventricle (Fig 4 D), vegetations and hemorrhages in the endocardium of the right auricle, and some infection around the joints The other animal showed vegetations on the heart valves and some small focal lesions in the myocardium There were also a few lesions in the kidney medulla and involvement of the joints and muscles

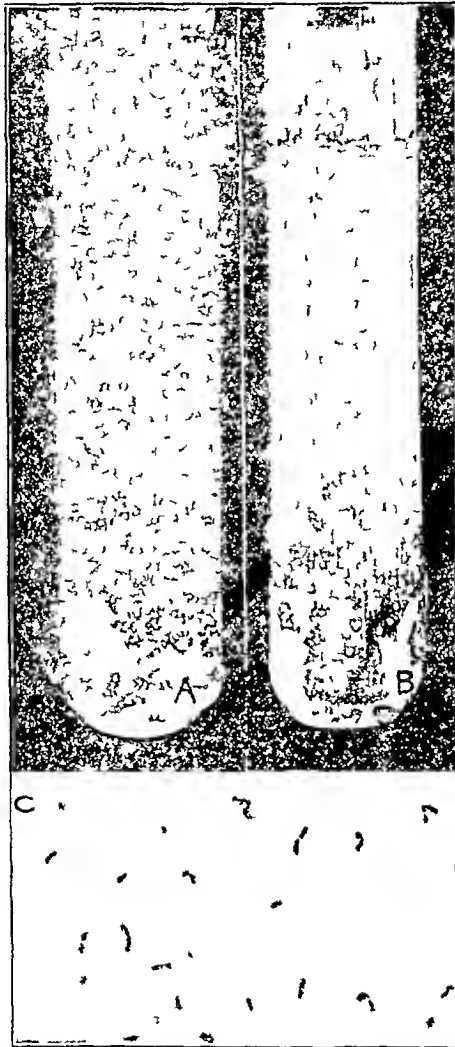


Fig 1—A, culture from periapical dental infection in glucose brain agar. Note the uniform growth of colonies throughout tube B, culture similarly made in which there is no growth at the top of tube C, photomicrograph of characteristic diplococcus from dental infection

Endocarditis and Auricular Fibrillation

CASE 3 *History*—L C H, a widow, aged sixty years, working as a clerk, complained of heart trouble. She had had chorea first at twelve years, with recurrent attacks for several years. At fourteen years, she had diphtheria and at twenty three, scarlet fever. Eight years before, she had had scleritis. For several years she had had albumen and pus in the urine. At one time, removal of a kidney was considered on account of the pyuria.

The patient stated she had been well up to 1912, eleven years before admission, when

he had a severe attack of influenza. Two weeks later he began to have arthritis, which persisted for six months. She was then well for several months, after which she began to have attacks of rapid and irregular heart. She had to give up work for seven weeks at this time on account of heart symptoms. About once a year, since this initial attack, she had had an attack of heart trouble incapacitating her for work for from six weeks to four months. During the past year the attacks had been occurring every few days lasting a few days at a time. The symptoms were worse on exertion. At times the ankles were swollen. Recently the patient had been to the Mayo Clinic where a diagnosis of par

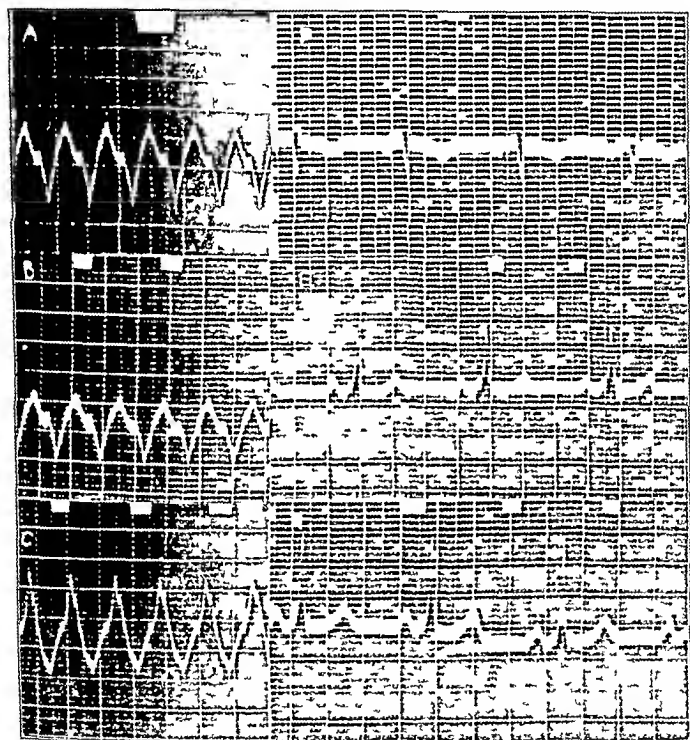


Fig. —A, B, and C electrocardiograms made from patient (Case 1) showing a tachycardia of ventricular origin. D, E, and F electrocardiograms taken one month later. Note the inverted T wave and change in QRS complex indicative of myocardial disease.

oxysmal auricular fibrillation was made. On admission there was a definite aortic insufficiency without demonstrable cardiac enlargement. The heart rate was slow and regular except for an occasional extra systole. The blood pressure was systolic, 140; diastolic, 70. The urine showed a few pus cells in clumps. There were 11 pulpless teeth, only 4 of which showed definite roentgenographic evidence of infection.

Animal inoculations.—The lower right second bicuspid and first and second molars were extracted first. All showed a profuse growth of nonhemolytic streptococci. Two rabbits were injected. One had, at necropsy, a few endocardial vegetations, a few abscesses in the

medulla of the kidney and a small amount of purulent fluid in the joint. The other rabbit showed a massive vegetative endocarditis of the tricuspid valve (Fig 5 A), a few lesions in the myocardium, and slight involvement of the joints. One rabbit was injected with the cultures from the lower left bicuspid and second molar. At necropsy, a few vegetations on the heart valves, numerous small abscesses in the wall of the left ventricle, a purulent arthritis, and a few kidney abscesses were found. Two rabbits were injected with the cul-

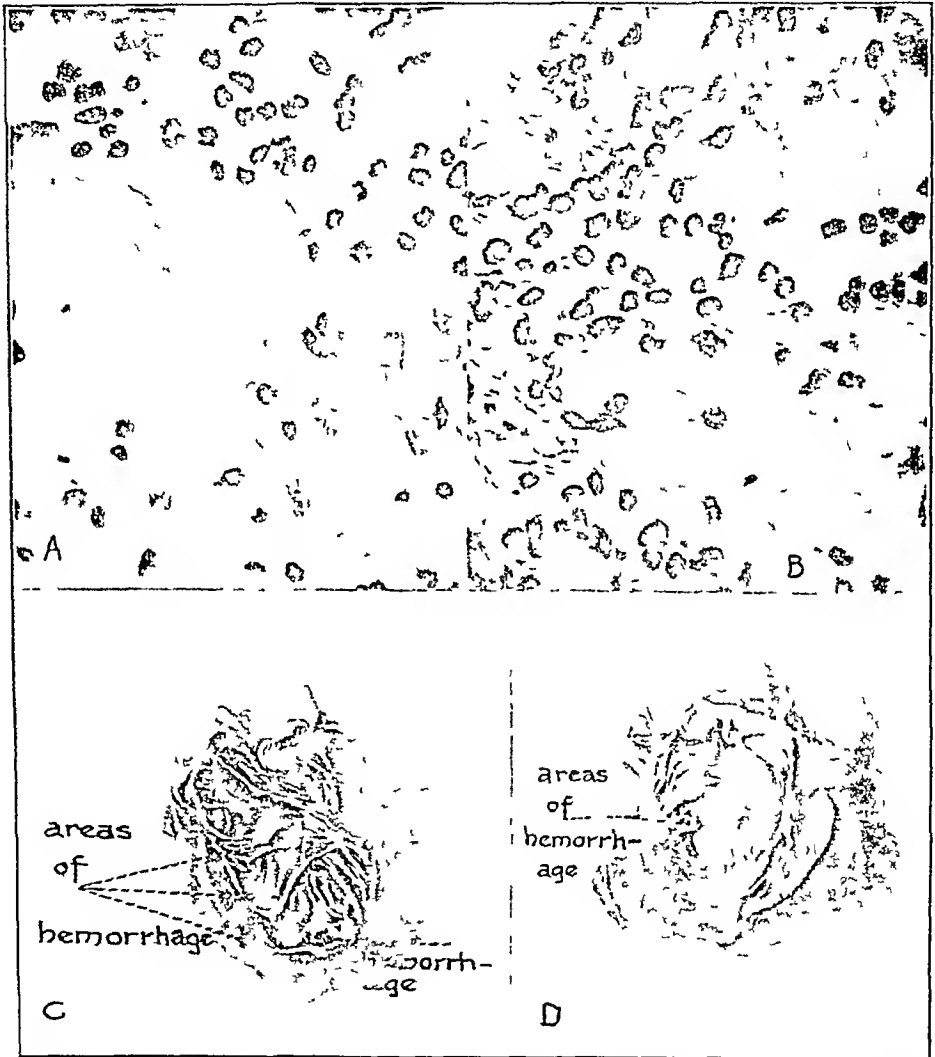


Fig 3—A and B sections of heart muscle obtained at autopsy (Case 1). Note the areas of cellular infiltration. Many of the cells are polymorphonuclear leucocytes. C and D, photographs of hearts of rabbits injected with streptococcus from infected third molar tooth. Note the areas of hemorrhage.

tures from the remaining teeth. One was dead the following day. There were many hemorrhages in the endocardium of the left ventricle and at the base of the papillary muscles. There were also a few hemorrhages and small vegetations in the right auricle near the ventricle. The other rabbit died two days after injection. At necropsy, only early vegetations on the mitral and the tricuspid valves, and mural thrombi in the right auricle were found.

Acute Phlebitis and Myocarditis

Case 4 History—J W P a physician, aged sixty years, had had a phlebitis of the left femoral vein in 1904 following an acute alveolar abscess. Following this there was frequent flare ups of the dental infection without further signs of systemic disease. In 1914 he began to have anginal attacks which continued to 1916. The attacks were entirely relieved by the removal of an infected tooth. In March 1923, the root of the bicuspid tooth became infected, and following this he had a recurrence of the phlebitis and anginal attacks. In June, 1923, nonhemolytic streptococcus was recovered from the blood. He became progressively worse, developed myocardial insufficiency and died. At autopsy there were multiple infarcts in the heart muscle.

Animal inoculation—After the extraction of the bicuspid root the infection of which had initiated the present illness, cultures were made from the socket and 2 rabbits were injected. The culture showed only a green producing streptococcus. The rabbits at autopsy showed only endocardial vegetations and infarcts of the myocardium. The upper right second and third molars were extracted in July, 1923. A profuse growth of streptococci was obtained from both. Two rabbits were injected. One was dead the following morn-

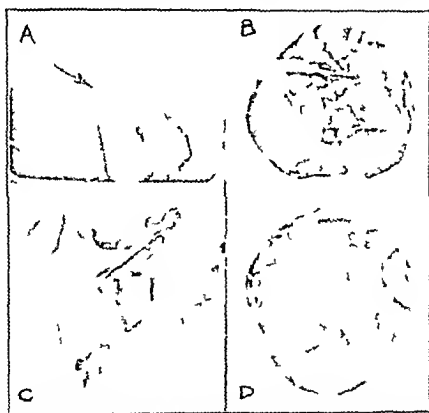


Fig. 4—A x ray negative pulpless tooth of patient (Case 4) which showed a profuse growth of streptococci on culture. B vegetations on tricuspid valve of rabbit injected with culture. C heart of another rabbit similarly injected showing multiple vegetations and hemorrhages in the wall of its auricle. D hemorrhage in myocardium.

ing. The autopsy revealed only multiple hemorrhages at the base of the valves. The second rabbit was dead forty-eight hours after injection. The examination showed only vegetations of the heart valve (Fig. 5 B).

Thromboangitis Obliterans

Case 5 History—J C W, a laborer aged forty-six years, complained of a painful great toe. For sixteen years he had suffered from pain in the calf of the leg on walking. For two years he had been able to walk only a short distance without resting. For one year he had had trouble in the great toe which he ascribed to an infected toe nail. Part of the toe had been amputated. On examination the end of the toe at the site of amputation was gangrenous. No pulse could be detected in the anterior or posterior tibial or the dorsalis pedis artery of either foot. The blood pressure was 130/80. The general examination was negative except for infected tonsils and extreme oral sepsis. The urine examination was negative. The hemoglobin was 90 per cent, the white count 12,100. The Wassermann was

negative. The blood chemical examination showed no deviation from the normal. The toe was first amputated, followed later by amputation of the foot.

Animal inoculations—Several teeth were extracted November 16, 1922. Two rabbits were injected. One showed only a meningitis. The other had at autopsy very large vegetations on the aortic valves (Fig 6). There were also lesions in the stomach, joints, and kidney.

Myocarditis

Case 6 *History*—A. L. S., a woman, aged fifty years, had been suffering from a myocarditis with a persistently rapid heart. The dental radiographs showed 4 pulpless teeth which were negative for infection. The cultures showed little growth in agar. From all broth cultures a streptococcus was obtained.

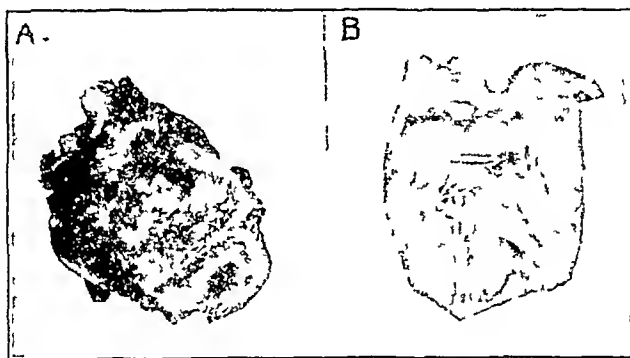


Fig 5—A, heart of rabbit injected with the culture from teeth of patient with aortic insufficiency and paroxysmal auricular fibrillation (Case 3). Note the large vegetations. B, heart of rabbit injected with the cultures from patient with myocarditis (Case 4).



Fig 6—Large vegetations on the aortic valves of heart of rabbit injected with cultures from patient (Case 5).

Animal inoculations—Four rabbits were injected intravenously, 1 animal died in a short while after inoculation, and 1 showed at autopsy, four days after the injection, a marked necrosis of the myocardium of the right ventricle, a second animal showed numerous hemorrhages in the heart muscle, and the third showed hemorrhages in the myocardium and vegetations in the tricuspid valve. These three animals showed some joint involvement, one had also a few lesions in the kidney medulla and in the muscles.

Acute Auricular Fibrillation

Case 7 *History*—J. M. R., a live stock dealer, aged fifty eight years, complained of palpitation of the heart. For a number of years he had suffered from chronic arthritis for which the tonsils had been removed, some teeth extracted, and the nasal sinuses drained.

There was now quite marked deformity of the joints but no evidence of active infection. For four or five years he had had attacks of rapid heart lasting only a short while. The present attack had begun the day before. There was moderate dyspnea on exertion. On examination the heart rate was 120-140 and totally irregular. The fluoroscopic examination showed marked dilation of the right side of the heart. There were no signs of valvular disease. The blood pressure was normal. The urine showed only a trace of albumen. Following the removal of the dental infection the heart rate returned to normal and has continued so for two years.

Animal inoculations.—The dental radiograph showed 2 roots (Fig 7 A) which were removed. The cultures showed a profuse growth of streptococci (Fig 7 B). Four rabbits were injected. Three animals at autopsy had hemorrhage in the myocardium. Two had vegetation in the valves. Two had also joint lesions, one an iritis and one hemorrhages in the stomach and duodenum.



Fig 7.—A radiograph of alveolar process showing two root tips remaining (Case 7). B culture from root tips. With this organism marked lesions in the heart of animals were produced.

Myocarditis with Angina Pectoris

CASE 8 History.—L. B. N., a carpenter, aged forty-two years, complained of recurrent attacks of pain over the heart. The attacks had begun eight months previously, were always brought on by exertion, and were relieved by rest. During one attack the left arm felt numb. He had always been well before the present illness. On examination numerous extra systoles were noted. The blood pressure was 100/75. There was no anemia. The blood Wassermann was negative. The urine showed no albumen, sugar or casts. There were numerous pulpless teeth. The tonsils were large and red. The fluoroscopic examination showed the heart and the aorta of normal size. The tonsils were removed and the pulpless teeth extracted. After the extraction of the teeth the patient felt so much better that he wished to return to work. Two weeks later he began to have dyspnea which increased in severity. He complained of a feeling of pressure in the chest and developed a pericardial friction rub. Signs of myocardial insufficiency soon developed followed by death about one year after the onset of symptoms.

Animal inoculations—Two rabbits injected with the broth culture from the tonsils showed no lesions of any kind. Three x ray positive teeth, the left lower first and second bicusps, the first molar were first extracted. All showed a profuse growth of streptococci. Two rabbits were injected and killed three days after inoculation. One showed only an arthritis with a pyelonephritis. The other showed a marked necrosis of the myocardium involving almost the entire right ventricle (Fig 8). A slight joint involvement was the only other lesion present.

One week later 4 more teeth were extracted. The left upper first bicuspid and lateral incisor were x ray positive and showed a profuse growth of streptococci on culture. The left upper was negative in the radiograph and showed also a profuse growth of streptococci. The left upper cuspid showed only a few colonies in the agar tube and was negative in the

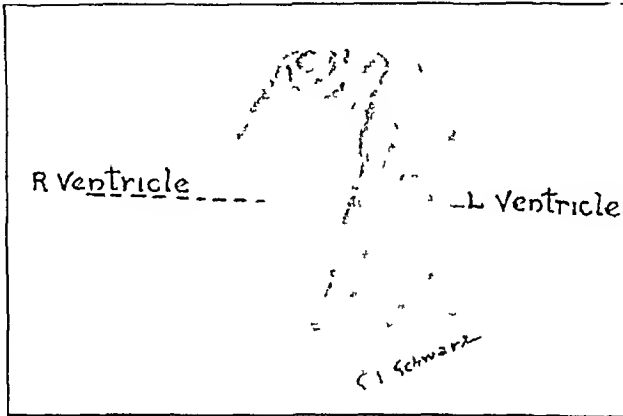


Fig 8—Massive necrosis of almost entire right ventricle of rabbit injected with a culture from the infected teeth of a patient suffering from myocardial disease (Case 8)



Fig 9—Multiple areas of necrosis in heart muscle and vegetation on the valve of a rabbit injected with the dental culture from a patient (Case 9)

radiograph. Two rabbits were inoculated with the mixed cultures. One showed at autopsy slight joint lesions and yellow plaques in the arch of the aorta. The other showed lesions in the joints and muscles, marked necrosis of the heart muscle, and several vegetations on the mitral valve.

Recurrent Acute Phlebitis and Arteritis

CASE 9 *History*—C. C. W., a telephone clerk, aged thirty-five years, complained of painful nodules in the leg. The first nodule had appeared at the age of twenty years and remained only a short while. He had had no further symptoms until the age of thirty when he had an attack of influenza. Four months after the attack a red, painful swelling appeared over the right popliteal vein. Following this there were many such swellings over

the veins of both lower extremities. Two years before, the right foot had become swollen and painful. One toe became gangrenous. A few months later the right leg was amputated.

On examination there was a small nodule above the internal condyle on one leg. The general examination was negative. The tonsils were small and deeply imbedded. The blood pressure was 90/58. The urine examination was negative. The white blood count was 8500, and the hemoglobin 85 per cent. The Wassermann test was negative.

Animal inoculations—The dental radiographs showed 6 pulpless teeth which were extracted. Four rabbits were injected with the cultures. Two showed at autopsy many

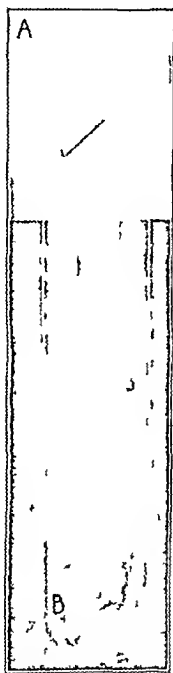


Fig 10—A area of edentulous bone with a residual infection (Case 10). B culture from curetting of area shown by arrow in A. Note the profuse growth of bacteria especially in the bottom of the tube.

areas of necrosis and hemorrhage in the myocardium and vegetations on the valves, (Fig 9). One had also areas of necrosis in the voluntary muscles, and both showed hemorrhages in the duodenum. One of the other rabbits showed no lesions, the other only muscle involvement.

Acute Arteritis

CASE 10 History—W M B, aged fifty five years, had had a number of serious infections of probable focal origin. Twenty years before he had had bilateral nevitis with complete loss of vision. At the same time a diagnosis of diabetes mellitus was made. In the following years he had suffered from chronic arthritis and a duodenal ulcer. On examination he was found to have a high grade secondary anemia, a marked arterial hypertension, and the urinary findings of chronic nephritis. Recently he had suffered from a swelling of

the leg and the foot. The swelling was limited to the lower half of the leg and the foot. There was no pulsation in the dorsalis pedis arteries. The condition seemed due to localized arterial disease. Gangrene seemed imminent.

Animal inoculations—The systemic diseases from which the patient had suffered were recognized as of focal origin but no definite foci had been found. Twenty years before all teeth had been extracted for extensive pyorrhea. The radiograph of the jaws shows numerous areas of apparent infection in the bone. It seemed quite evident that the anatomic changes in the patient were so extensive that removal of foci could be of little value. One suspicious area (Fig 10 A) was exposed and curetted. The bone was quite soft. The culture showed a profuse growth of streptococci which would not grow to the top of the brain broth agar tube (Fig 10 B).

Two rabbits were injected with the culture. Both animals developed a mild iritis. One at autopsy showed no other lesions. The other showed endocardial vegetations, abscesses in the kidney and marked joint involvement.

REFERENCES

- ¹Rosenow, E. C. Jour Amer Med Assn, 1915, 11, 1687
- ²Haden, R. L. Arch Int Med, 1923, 33, 828
- ³Haden, R. L. Am Jour Med Sc, 1925, 61, 407
- ⁴Haden, R. L. Arch Int Med, 1925, 35, 457
- ⁵Rosenow, E. C. Jour Am Med Assn, 1915, 11, 1687
- ⁶Detwiler, H. K., and Robinson, W. L. Jour Am Med Assn, 1916, 13, 1653
- ⁷Thalhimer, W., and Rothschild, M. A. Jour Exper Med, 1914, 11, 444
- ⁸Henrici, A. T. Jour Infect Dis, 1916, 11, 572

THE SUGAR AND CHLORIDE CONTENT OF THE CEREBROSPINAL FLUID WITH SPECIAL REFERENCE TO NEUROSYPHILIS

By S. WILLIAM BLOCKER M.D., ROCHESTER, MINN.

THE reducing substance in the cerebrospinal fluid in man has received a certain amount of attention by chemists and clinicians in the last two decades. It has been shown to be essentially a monosaccharid, probably glucose. The normal amount has been variously estimated by different authors, from a minimum of 40 mg for each 100 cc to a maximum of 134 mg. In contrast to this discrepancy Mestrezat who has done considerable work on the sugar of the cerebrospinal fluid which he believes is allied to if not identical with the true and dialyzable sugar of the blood plasma, gives the normal content as from 55 to 65 mg for each 100 cc, with an average of from 59 to 60 mg. Most authors grant a much greater range of normal variation. Thalheimer and Updegraff, by means of the Folin and Wu method, estimated the upper limit of normal at from 60 to 65 mg. Kelley pooled normal fluids and obtained 55 mg by the same method. The variation in results is probably attributable to difference in method of estimating sugar and precipitating the blood protein. The herculean task of comparing a score or more of reported procedures would be necessary to determine this. A few authors have made parallel determinations by two or three methods, with some interesting observations. For instance, Stevenson found the discrepancy between values obtained by Folin's and Shaffer's methods was greater in patients with encephalitis than in normal persons. He considered this probably due to substances capable of reducing Folin's but not Shaffer's reagent.

The amount of reducing substance has been found to be abnormal in cases of acute and chronic meningitis, encephalitis and diabetes. A moderate amount of work has been done on the cerebrospinal fluid in cases of neurosyphilis. In cases of undifferentiated neurosyphilis increased values were found by Boyd, Veran and Vernet, Kaplan, and Csaki, normal values were reported by Rieger and Solomon, Kahler, and Kraus and Corneille, and decreased values were found by Borberg, Holzmänn, Kelley, and Martin. In cases of tabes dorsalis increased amounts were noted by Lowy, Kaplan, Csaki, and Polonowski and Duhot, normal values were found by Kahler, and Rieger and Solomon, and decreased amounts were reported by Borberg and Holzmänn. In cases of general paresis high values were found by Csaki, normal values by Briand, Marcel and Rouquier, and Rieger and Solomon, and decreased values by Borberg, Holzmänn, Kelley, and Polonowski and Duhot. Alpers, Campbell and Prentiss found an average of 65 mg in cases of paresis without treatment, and 55 mg in cases in which treatment had been given, Weston found an average of 71.8 mg in the cases without treatment and

72.5 mg in the cases with treatment. Thus it is seen that there is no unanimity of opinion, but each individual author's findings are essentially the same for the three phases of neurosyphilis. Hopkins, Stevenson, and Weil have reported varying results. Hopkins states that lower figures are often found in cases of syphilis than in any other disease, with the exception of non-syphilitic meningitis.

The relation of whole blood sugar to cerebrospinal sugar has been studied by a few workers. Bang states that they are present in the same percentage. Hopkins, using Bang's method, found the blood sugar to be 10 mg higher. Polonowski and Duhot found the ratio of cerebrospinal fluid to blood sugar to be equal to 1:1. Scivantie states that the ratio varies from 0.4:1 to 1:1. Myers and Fine studied the spinal fluid in 15 cases in which there were varying degrees of nitrogen retention, and found the sugar of the cerebrospinal fluid to be 57 per cent of that in the blood. In cases without diabetes Rusznyák and Csáki found the sugar in the spinal fluid to be much less than that of the blood plasma, the average difference being 54 mg. This might be construed as contradictory to Mestrezat's theory, which has already been mentioned. Many investigators did not consider the blood sugar at all, and made no attempt to control the alimentation of their patients. Polonowski and Duhot found higher values when the spinal fluid was withdrawn from three to five hours after the last meal than when it was withdrawn ten hours after the last meal. Increased values have been found in the cerebrospinal fluid in cases of diabetes. Thalhimer and Updegraff could find no increase, however, until the blood sugar reached 190 mg and believed this might signify a threshold level for passage of sugar into the spinal fluid. They estimated the value of the sugar in the spinal fluid at 45 per cent of that found in the blood. Normal blood sugar is generally given as ranging from 90 to 120 mg for each 100 c c of blood.

TECHNIC FOR ESTIMATION OF SUGAR CONTENT

In this study the concentration of sugar, both in the blood and cerebrospinal fluid, was determined by the Rothberg and Evans' modification of the Folin and Wu method. This method was chosen with a view to accurate determination, and the work was personally performed. The blood protein was precipitated as recommended by Folin and Wu, but for the cerebrospinal fluid the procedure was altered. To 20 c c of fluid were added 60 c c of distilled water, 10 c c of 10 per cent sodium tungstate, and 10 c c of two-thirds normal sulphuric acid. This is considered sufficient to precipitate all the protein and permits the use of the customary standard No. 1 of the Folin and Wu method for both blood and spinal fluid, thereby eliminating the preparation of a standard of one-half this strength, as suggested by Foster. The unknown is diluted until the intensity of color matches that of the known. This is generally obtained at 30 c c.

The blood and spinal fluid of the normal subjects were collected simultaneously about sixteen hours following the last meal. This was also true of most of the patients who had syphilis without involvement of the central nervous system. When the central nervous system was affected the spinal

TABLE I

INITIAL CONCENTRATION OF SUGAR IN THE SPINAL FLUID AND BLOOD IN 175 CASES

TYPE OF CASE	SPINAL FLUID				BLOOD		REMARKS
	CASES	RANGE OF SUGAR CONCENTRATION MG	AVERAGE SUGAR CONCENTRATION MG	CASES	RANGE OF SUGAR CONCENTRATION MG	AVERAGE SUGAR CONCENTRATION MG	
Controls (non syphilitic)	1	56 to 77	67	19	84 to 97	90	Hypoglycemia (?)
	1		45	1		66	
Latent syphilis	10	53 to 69	61	10	80 to 103	90	Pancreatitis
	1		100	1		276	
Primary and secondary syphilis	4	59 to 70	64	4	81 to 90	86	
Congenital syphilis	1		56	3		90	
Vascular neurosyphilis	2	52 to 54	53	2	70 to 136	10	
Serologically negative neurosyphilis	1		63	1		97	
Undifferentiated neurosyphilis	37	45 to 71	58	32	60 to 129	90	Glycosuria several times
	1		82	1		109	
Asymptomatic neurosyphilis	12	42 to 68	56	11	67 to 122	100	
Asymptomatic meningel neurosyphilis	12	47 to 74	62	9	59 to 126	94	
Tabes dorsalis	59	41 to 73	57	50	64 to 156	93	
Juvenile paresis	1	40 to 53	50	2	78 to 84	81	
General paresis	9	41 to 67	57	7	78 to 105	94	

fluid was removed at the time intraspinal treatment was given (Table I). This followed the administration of arsphenamine on the previous day and the ingestion of a light breakfast as a rule on the morning of intraspinal treatment. The length of time without food made some difference in the content of sugar in the blood, but no alteration in the content of sugar in the spinal fluid could be ascertained.

Precipitation was carried out very soon after the spinal fluid was withdrawn. If the sugar could not be determined immediately, a few drops of toluol were added to the filtrate and it was placed in a refrigerator. This precaution was taken to avoid possible glycolysis, as Csáki reported 50 per cent loss of reducing substance in three hours at room temperature in the case of cerebrospinal fluid from a patient with diabetes. Lowy found no decrease in from twenty four to forty eight hours at room temperature and at 37° C. Stevenson obtained the same result after the fluid had stood for two or more days. The last two authors were not considering the spinal fluid in cases of diabetes.

The supposedly normal patient with low concentration of sugar in the blood and spinal fluid had no demonstrable abnormality except fibromyoma of the uterus. This was the only case in the entire series with subnormal findings in both blood and spinal fluid. This condition has been experimentally produced by Polonowski and Duhot as will be mentioned later. At operation

and necropsy pancreatitis was discovered in the case of latent syphilis with high concentration of sugar in the blood and spinal fluid. It is interesting to note that urinalyses, taken up to five days before sugar determinations were made, showed no sugar. In the case of neurosyphilis with high concentration of sugar in the spinal fluid and normal concentration in the blood there was a slight trace of sugar in the urine on several occasions. This patient was Jewish, and there may have been some disturbance in carbohydrate metabolism, although the glycosuria was thought to be of renal origin. In the cases without syphilis, used as controls, there was no demonstrable organic lesion of the nervous system.

RELATION OF THE CONCENTRATION OF SUGAR IN THE SPINAL FLUID TO SEROLOGIC FINDINGS

Correlation of the concentration of sugar in the spinal fluid in cases of neurosyphilis with serologic findings has been attempted by a few workers. Borberg found especially low values in cases with pleocytosis. Kahler states that the cell count makes no difference, although he had no cell count above 55 lymphocytes for each cubic millimeter. Moates and Keegan found no connection between the sugar content and the other findings. Wittgenstem states that many factors must be considered in the interpretation of results and presents noteworthy tables. In general he assumes that in cases of tabes dor-

TABLE II
CONCENTRATION OF SUGAR IN THE SPINAL FLUID IN CASES OF NEUROSYPHILIS

TYPE OF CASE	HIGH CELL COUNT*, MG	NORMAL CELL COUNT, MG	COLLOIDAL BENZOIN REACTION IN FIRST ZONE, MG	COLLOIDAL BENZOIN REACTION IN OTHER ZONES, MG
Vascular neurosyphilis		56	56	
Undifferentiated neurosyphilis	56	59	54	60
Meningeal neurosyphilis	64	64	64	64
Asymptomatic neurosyphilis	60	59	52	59
Tabes dorsalis	53	58	54	57
Juvenile paresis		53	53	
General paresis (1 case)	49	60	62	51

*More than 10 cells for each cubic millimeter was considered abnormal.

salis and undifferentiated neurosyphilis, a high cell count with low concentration of sugar in the spinal fluid denotes a meningitic process, that a high cell count in cases of tabes dorsalis with high concentration of sugar denotes a complicated cerebral process, and that a high cell count in cases of neurosyphilis with high concentration of sugar may be due to allergy. He supports this assumption by stating that in one such case monoplegia developed during treatment with arsphenamine. He states that the sugar content seems to have diagnostic significance in cases of cerebral inflammation, in which he finds higher percentages. His tables must be studied to be appreciated. In Table II are shown the results of my cases with respect to cell count and col-

loidal benzoin reaction. The percentages represent the average concentration of sugar in the spinal fluid for the various groups.

* There is a slight tendency to lower values along with high cell count, and also with colloidal benzoin reactions in the first zone.

INFLUENCE OF ALIMENTARY HYPERGLYCEMIA

Since it was found by Hess and Potzl that the oral ingestion of iodides was followed by their appearance in the cerebrospinal fluid, only in cases of meningitis, since increased iodide values in cases of syphilitic meningitis were found by Osborne, and since increased nitrate values, after administration of the salt, were found by Mestrezat and Grigoux, studies were made of the effect of alimentary hyperglycemia on the sugar in the spinal fluid in cases of neurosyphilis. Kelley increased the blood sugar of rabbits by intravenous injection of glucose and found no increase in the sugar in the spinal fluid. Polonowski and Duhot produced hyperglycemia by subcutaneous injection of epinephrin and produced an increase in the sugar in the spinal fluid. They considered, however, that the epinephrin may have introduced an added factor by possibly altering the permeability of the choroid plexus. They produced alimentary hypoglycemia by administering large amounts of glucose and saccharose, and found a decrease in the sugar in the fluid. I first determined the sugar in the blood and spinal fluid to determine the patient's normal. Two weeks later I administered orally 100 gm of glucose fifteen minutes, thirty minutes, or one hour before the withdrawal of blood and spinal fluid, using the various intervals on different occasions. The blood and spinal fluid were withdrawn almost synchronously. Glucose was given to 21 patients for a total of 30 administrations. The content of sugar in the blood was highest after the half hour interval, reaching a peak of 186 mg, but without a definite increase in the sugar in the spinal fluid at any time. In 2 cases there was no change, in 15 cases there was an average increase of 6 mg and in 13 cases an average decrease of 5 mg. The greatest increase, 10 mg, was in a case of meningitis. The general averages of various types of neurosyphilis were approximately identical. The highest concentration of sugar in the blood, 186 mg, was associated with an increase of but 5 mg. The negative result may be due to the fact that the threshold value of 190 mg as found by Thalheimer and Updegraff, was not reached.

INFLUENCE OF TREATMENT

A majority of the patients with neurosyphilis had had a certain amount of treatment, but there was no appreciable difference in the findings in these cases and those untreated. The patients were being treated by intravenous and intraspinal injections of arsphenamine by the Swift-Ellis-Ogilvie method, with iodides, and mercury or bismuth. To determine the behavior of the sugar in the spinal fluid under treatment, determinations were made on 2 occasions in 24 cases, and on 3 occasions in 2 cases. This is exclusive of patients who received glucose. The determinations were made at intervals of from two weeks to five months. In 1 case the result was the same at 2 examinations, in 15 cases there was an average increase of 5 mg, and in 8 cases

there was an average decrease of 7 mg. In the 2 cases with 3 examinations (at intervals of two weeks) the variation in results did not exceed 3 mg. The length of time between examinations seemed to make no difference. Kelley found an average concentration of sugar in the spinal fluid of 21 mg. in cases of neurosyphilis without treatment and an average of 62 mg. in cases in which treatment was given. Biach, Kerl and Kahler found from 40 to 90 mg. in cases without treatment and as high as 340 mg. in a case following treatment. These authors did not state specifically that the figures before and after treatment were obtained in the same cases. Wittgenstein obtained marked increase in sugar in the spinal fluid in cases of neurosyphilis after intravenous injection of neoarsphenamine, with and without spinal drainage. Alpers, Campbell, and Pientiss found the content decreased in cases of paresis following treatment, and Weston found slightly higher values.

PROGNOSTIC VALUE OF THE DETERMINATION OF SUGAR IN THE SPINAL FLUID

This phase of the subject has received scant attention. Wittgenstein believes increased values for sugar in the spinal fluid signify cerebral involvement, which might increase the gravity of the situation. If the colloidal benzoin reaction in the first zone signifies cerebral parenchymatous involvement, as Osborne believes, my findings are at variance with those of Wittgenstein. My patients have been observed as long as one and one-half years. Those who on the basis of clinical and serologic findings promise to be resistant to treatment tend to have a decreased concentration of sugar in the spinal fluids with a greater range in values. There is, however, no apparent difference between those with low and those with high values. Further observation will be necessary to determine the prognostic value of these levels.

DISCUSSION

There are several sources of error in making determinations, some of which have been mentioned by Polonowski and Duhot: the blood which is examined is from the peripheral vessels and may differ from that in the choroid plexus, the determinations have been made on whole blood and not on plasma, and the filtrate may contain reducing substances other than sugar. In fact, Mestiezat goes so far as to condemn all methods of precipitating blood protein except by means of mercuric acid sulphate. Due to the small variation, the method of determining sugar must be as accurate and sensitive as possible. Division of cases of neurosyphilis into distinct groups is inaccurate on account of the manifold involvement. Comparison of the sugar content of the spinal fluid with cytologic and serologic findings is inaccurate, since a high cell count may fall markedly and a colloidal benzoin reaction in the first zone may disappear under intraspinal medication with little or no change in the sugar content.

With regard to the etiology of decrease in the sugar content of the spinal fluid, it is possible that, as suggested by Wilcox and Lyttle, disease of the meninges or choroid plexus may prevent the glucose from entering the spinal fluid. The varying results could be explained by difference in the degree of involvement of the portion of the meninges that covers the choroid plexus. It would be interesting to study the choroid plexus at necropsy in

cases of neurosyphilis in relation to degree of involvement and also in regard to glycogen content, to which attention has been directed by Yoshimura Kelley, on the basis of inoculation experiments *in vitro*, believes that the spirochete utilizes the sugar of the spinal fluid as food. The difficulty encountered in demonstrating the organisms in the spinal fluid would more or less preclude their presence in sufficient numbers to make any appreciable change in the concentration of sugar.

CHLORIDES OF THE CEREBROSPINAL FLUID

There are fewer reports of examination of the chlorides of the cerebrospinal fluid than of the determination of sugar, and less variation in the estimation of normal values. The results range from a minimum of 610 mg to a maximum of 750 mg for each 100 cc of spinal fluid. The normal chlorides of whole blood range from 550 to 600 mg for each 100 cc. These figures are

TABLE III
CONTENT OF CHLORIDES IN SPINAL FLUID AND BLOOD

TYPE OF CASE	CASES	SPINAL FLUID		BLOOD	
		RANGE OF CHLORIDES MG	AVERAGE AMOUNT OF CHLORIDES, MG	RANGE OF CHLORIDES MG	AVERAGE AMOUNT OF CHLORIDES MG
Normal	11	710 to 775	731	440 to 460	480
Latent syphilis	8	710 to 740	727	460 to 620	510
Primary and secondary syphilis	5	710 to 738	723	420 to 520	455
Congenital syphilis	1		730	—	480
Undifferentiated neurosyphilis	37	660 to 750	739	430 to 670	513
Asymptomatic neurosyphilis	21	660 to 760	730	426 to 620	522
Tabs dorsalis	41	685 to 775	734	410 to 620	510
General paresis	4	730 to 175	739	440 to 520	485

expressed in terms of sodium chloride. Flockenhaus and Fonseca found them as high as 900 mg in cases of paralysis. Csaki found them increased in cases of syphilis and "metasyphilis." He observed decreased values in one case of syphilitic meningitis, which increased following treatment. Depisch and Richter Quittner found increased values in cases of syphilis.

TECHNIC FOR THE ESTIMATION OF CHLORIDE CONTENT

Estimation of chlorides was made on the protein free filtrates such as were used in determining the content of sugar. The following is the method employed.* The silver nitrate reagent is prepared by dissolving exactly 2.905 gm of silver nitrate in about 100 cc of water and dissolving 30 gm of ferrie ammonium sulphate in about 300 cc of water, and by adding these solutions together, with 500 cc of concentrated nitric acid, in a 1000 cc volumetric flask, and by diluting to the mark with water, 1 cc equals 1 mg of sodium chloride.

*The method is that of Whitehorn as modified by W. G. Karr, Chief Chemist, Philadelphia General Hospital.

The ammonium thiocyanate reagent is prepared by dissolving about 27 gm of ammonium thiocyanate in about 1 liter of water. This is placed in a 5 c c microburette. In a 50 c c porcelain casserole are placed 10 c c of water and 10 c c of the silver nitrate reagent. This is then titrated with the thiocyanate from the burette. The end-point is the first reddish-brown color that persists for fifteen seconds. This titration is repeated for confirmation. Two hundred times the amount of thiocyanate solution required for the titration is diluted to 1 liter, 5 c c of this solution is then equal to 10 c c of the silver reagent and is equivalent to 10 mg of sodium chloride.

Ten cubic centimeters of the protein-free filtrate is transferred to a 50 c c porcelain casserole and 10 c c of the silver nitrate reagent added. It is allowed to stand five minutes and then is titrated with the ammonium thiocyanate solution from the microburette until a slight reddish-brown persists for at least fifteen seconds.

If r = quantity of thiocyanate used (in cubic centimeters)
 and λ = quantity of sodium chloride (in milligrams for each 100 c c of blood)
 then $\kappa = 200 (5-r)$

In the case of the cerebrospinal fluid the filtrate must be diluted by an equal amount of water or the results divided by two, since the filtrate is twice as concentrated as that from the blood.

This method was chosen because it permitted determinations to be made on the same filtrate as was used for the estimation of sugar. One hundred thirty-one determinations were made on the blood and spinal fluid of 100 patients. There was a little variation from time to time, the following being a typical example of a case of meningeal neurosyphilis.

DATE	WHOLE BLOOD CHLORIDES, MG	SPINAL FLUID CHLORIDES, MG
June 10, 1924	560	740
June 24, 1924	580	755
July 8, 1924	520	730

In general, the range of variation is greater in the blood than in the cerebrospinal fluid, as is evident in Table III which includes cases both with and without treatment. Specimens were examined at different times during treatment, and all results were within normal limits.

SUMMARY

There is a definite but small decrease, with rather wide range of values, in the reducing substance of the cerebrospinal fluid in cases of neurosyphilis, as determined by Rothberg and Evans' modification of the Folin and Wu method. Simultaneous determination of blood sugar is of assistance in a small percentage of cases. There is a slight normal variation in the content of sugar in the spinal fluid but not as great as in blood sugar. Length of time without food made some difference in the level of sugar in the blood, but apparently none in that of the cerebrospinal fluid.

There is a slight tendency toward low values for sugar in the spinal fluid along with high cell count and in cases with colloidal benzoin reaction in the first zone. The regular comparative tabulations of Wittgenstein could not be

duplicated. Alimentary hyperglycemia up to 185 mg produced no increase of sugar in the spinal fluid. There was no alteration in sugar content under treatment in cases followed as long as five months. The extremely low values for sugar in the spinal fluid in cases of neurosyphilis and the marked rise following treatment, as noted by some authors, could not be confirmed. No information was obtained that would definitely aid in prognosis although observation has lasted one and one half years. Careful observation over years with repeated determinations of the content of sugar in the spinal fluid may prove valuable.

There is a variation in the chloride content of the cerebrospinal fluid which is not as great as in the chlorides of the whole blood. In all the cases of neurosyphilis, the spinal fluid showed a normal chloride content.

REFERENCES

- 1 Alpers, B. J., Campbell, C. J. and Prentiss, A. M. The Spinal Fluid Sugar. *Arch Neurol and Psychiat* 1924 xi 653-663
- 2 Bang, Ivar. Quoted by Lowy
- 3 Biach, P., Kerl, W. and Kahler, H. Zur Kenntnis der Veränderungen der Spinalflüssigkeit nach Neurolysenanwendung. *Wien klin Wchnschr* 1914 ii 1098-1101
- 4 Borberg. Quoted by Kahler
- 5 Boyd, W. The Cerebrospinal Fluid in Certain Mental Conditions. *Jour Ment Sc* 1912 lxxii 203-225
- 6 Briand, M., Marcel and Rouquier, A. Quoted by Kahler
- 7 Csáki, Ladislaus. Ueber den diagnostischen und prognostischen Wert der Bestimmung des Chlor- und Zuckergehaltes und des Refraktionswertes im Liquor cerebrospinalis mit besonderer Rücksicht auf Meningitis. *Ztschr f klin Med* 1924 c 271-281
- 8 Dupisch, F. and Richter, Quittner, M. Ueber die chemische Zusammensetzung des menschlichen Liquor cerebrospinalis. *Wien Arch f inn Med* 1923 i 321-344
- 9 Flockenhauer, Max and Fonseca, Fernando. Beiträge zur Liquordiagnostik. *Deutsch med Wchnschr*, 1924 ii 1045-1046
- 10 Folm, Otto and Wu, Hien. A System of Blood Analysis. *Jour Biol Chem* 1919 xxxviii, 81-110
- 11 Foster, H. E. Hyperglycorrhachia in Epidemic Encephalitis. *Jour Am Med Assn* 1921 lxxvi, 1300-1301
- 12 Hess, L. and Potzl, O. Ueber Schwankungen im Zuckergehalt des Liquor cerebrospinalis bei psychischen Erkrankungen. *Wien klin Wchnschr* 1910 xxiii 1065-1069
- 13 Holzmann. Quoted by Kahler
- 14 Hopkins, A. H. The "Sugar" Content of the Spinal Fluid in Meningitis and other Diseases. *Am Jour Med Sc* 1915 cl 847-857
- 15 Kahler, H. Ueber Veränderungen des Zuckergehaltes in der Cerebrospinalflüssigkeit bei inneren und Nervenkrankungen. *Wien klin Wchnschr* 1922 xxxv 8-11
- 16 Kaplan, D. M. Analyse der Spinalflüssigkeit und des Bluteserums in ihrer Bedeutung für die Neurologie. *Deutsch med Wchnschr* 1913 i 1035-1038
- 17 Kelley, A. G. Sugar Findings in Normal and Pathological Spinal Fluids. *South Med Jour* 1923, xvi 407-411
- 18 Kraus, W. M. and Cornille, G. G. Sugar Content of the Cerebrospinal Fluid in Health and Disease. *JOUR LAB AND CLIN MED* 1916 i 685-687
- 19 Lowy, Julius. Ueber die reduzierenden Substanzen des Liquor cerebrospinalis. *Ztschr f klin Med* 1910, lxxxiii 285-290
- 20 Martin, W. B. Variation in the Sugar Content of Spinal Fluid. *Virginia Med Month* 1922 xlviii, 689-691
- 21 Mestrezat, W. Glycorachie à propos de la note de M. Servantie. *Compt rend Soc de biol*, 1923 lxxxix 289-291
- 22 Mestrezat, W. Glycorachie normale à propos de la note de MM. Fontanel et Leulier. *Compt rend Soc de biol* 1924 xc 339-341
- 23 Mestrezat, W. and Gaujoux, E. Présence de Nitrates et de Nitrites dans le Liquide Céphalo-rachidien. Perméabilité méningée aux Nitrates. *Compt rend Soc de biol* 1909 lxxvi 424-426
- 24 Mestrezat, W. and Gaujoux, E. Exagération de la perméabilité méningée aux nitrates. Diagnostic de la méningite tuberculeuse. *Compt rend Soc de biol* 1909 lxxvi 533-535
- 25 Montes, G. H. and Keegan, J. J. Method and Diagnostic Value of Quantitative Determination of Sugar in the Cerebrospinal Fluid. *JOUR LAB AND CLIN MED* 1923 vii, 825-828

- 26 Myers, V C, and Fine, M S Comparative Distribution of Urea, Creatinine, Uric Acid, and Sugar in the Blood and Spinal Fluid, Jour Biol Chem, 1919, xxxvii, 239 244
- 27 Osborne, E D Iodin in the Cerebrospinal Fluid, with Special Reference to Iodid Therapy, Jour Am Med Assn, 1921, lxxvi, 1381 1386
- 28 Osborne, E D Clinical and Serologic Value of the Colloidal Benzoin Reaction, Arch Dermat and Syph, 1925, xii, 706 719
- 29 Polonowski, M, and Duhot, E Dosage du Sucre dans le Liquide Céphalorachidien, Compt rend Soc de biol, 1921, lxxiv, 600 602
- 30 Polonowski, M, and Duhot, E Sucre libre du Sang et du Liquide Céphalorachidien, Compt rend Soc de biol, 1921, lxxiv, 687 688
- 31 Polonowski, M, and Duhot, E Glycémie et glycorachie, Presse méd, 1923, i, 60 62
- 32 Rieger, J B, and Solomon, H C Spinal Fluid Sugar, Boston Med and Surg Jour, 1916, clxv, 817
- 33 Rothberg, Vera E, and Evans, F A A Modified Folin and Wu Blood Sugar Method, Jour Biol Chem, 1923 1924, lvm, 443 451
- 34 Rusznjak St, and Csiki, L Beziehungen zwischen dem Zuckergehalt des Plasmas und des Liquors, Biochem Ztschr, 1922, cxxviii, 355 357
- 35 Servantie, L Glycorachie et Rapport Hémoméningé, Compt rend Soc de biol, 1923, lxxix, 167 168
- 36 Stevenson, L D A Comparative Study of the Sugar Content of the Spinal Fluid in Diseases of the Nervous System, Arch Neurol and Psychiat, 1921, vi, 292 294
- 37 Thalheimer, William, and Updegriff, Helen The Sugar Content of the Blood and Spinal Fluid in Epidemic Encephalitis, Arch Neurol and Psychiat, 1922, viii, 15 23
- 38 Verain, M, and Vernet, P Quoted by Kahler
- 39 Weil, M P Les Hyperglycorachies non Diabétiques, Ann de méd, 1918, v, 463 489
- 40 Weston P G Sugar Content of the Blood and Spinal Fluid of Insane Subjects, Jour Med Research, 1916, xxv, 199 208
- 41 Wilcox, H B, and Lyttle, J D The Diagnostic Value of Sugar Concentration in Spinal Fluid, Arch Pediat, 1923, vi, 215 225
- 42 Wittgenstein, A Zur klinisch diagnostischen Bewertung des Zuckergehalts der Zerebro spinalflüssigkeit, Deutsch med Wchnschr, 1923, i, 246 248
- 43 Yoshimura Quoted by Hess and Potzl

THE USE OF ISOPROPYL ALCOHOL IN THE PREPARATION OF WASSERMANN ANTIGENS*

BY ROBERT M ISHAM, PH D, OKMULGEE, OKLA

COMPARATIVELY recently isopropyl alcohol has become available in quantity at reasonable prices. Because of the high tax on ethyl alcohol and the onerous restrictions connected with its withdrawal and use, it is in many instances being replaced by isopropyl alcohol, which is less costly and is free from all regulation, since it is nonpotable.

These considerations have led the author to undertake an investigation of the possibility of preparing Wassermann antigens by use of isopropyl alcohol instead of ethyl alcohol. The close similarity in physical properties of the two alcohols indicated the probable success of the investigation, while the results obtained show isopropyl alcohol to be actually superior to ethyl alcohol for this purpose. It proved to be a better solvent for the antigenic substances of normal tissue than ethyl alcohol.

In this work two types of antigen were prepared: the simple alcoholic extract of heart muscle and the alcohol ether soluble and acetone insoluble antigen as used by the Hygienic Laboratory. Results were controlled by

*Received for publication February 3 1926

making identical preparations from the same hearts, using 95 per cent ethyl alcohol for the extraction

The simple alcoholic extract prepared by use of isopropyl alcohol was much higher in antigenic power than that prepared by use of ethyl alcohol. While it required twenty five antigenic units to give partial inhibition of hemolysis with normal sera, in the case of the antigenic extract prepared with isopropyl alcohol, only ten units were required in the case of the ethyl alcoholic extract.

In the preparation of the acetone insoluble antigens, isopropyl alcohol was found to give approximately a 10 per cent greater extraction of acetone insoluble material than ethyl alcohol. The antigenic power of the methyl alcohol and ether solutions of the acetone precipitates was equal in the two cases. Even 0.20 cc of the antigen prepared with isopropyl alcohol failed to give any inhibition of hemolysis with normal sera, while some inhibition was apparent with 0.16 cc of the antigen prepared with ethyl alcohol.

EXPERIMENTAL

Simple Alcoholic Extracts—A fresh beef heart was freed from fat and connective tissue and the heart muscle ground in a meat grinder. The ground muscle was thoroughly mixed, and two 200 gm portions were weighed out. These were transferred to 1200 cc flasks and extracted for two weeks at 37° C, the one with one liter of 91.1 per cent isopropyl alcohol, the other with one liter of 95 per cent ethyl alcohol. During extraction the flasks were well shaken, three times daily.

After extracting for two weeks, the solutions were filtered and the filtrates preserved in tightly stoppered bottles in the ice box. On cooling, both solutions deposited some material, the isopropyl alcohol solution depositing considerably more than the ethyl alcohol solution. The isopropyl alcohol solution retained a considerably higher color than the ethyl alcohol solution.

These simple alcoholic extracts were tested for antigenic and anticomplementary properties in the usual manner in an antsheep hemolytic system by using two units of complement, two units of amboceptor, and pooled strongly positive and pooled normal sera respectively with graded amounts of antigen. Incubation for fixation of complement was at 37° C, for thirty minutes. After adding amboceptor and sheep cells, incubation at 37° C was continued for thirty minutes, and the tubes were then allowed to stand overnight at 15° C before reading. Results of these tests appear in Table I.

A comparison of the data present in Table I shows that even 0.004 cc of the isopropyl alcohol antigen gives total inhibition of hemolysis with positive sera, while no inhibition with normal sera appears until amounts in excess of 0.10 cc of the antigen are used. We thus have a ratio of 0.10/0.004 equals 25/1.

In the case of the ethyl alcohol antigen it requires 0.01 cc to give total inhibition with positive sera, and amounts above 0.10 cc show inhibition with normal sera. The ratio here is thus only 0.10/0.01 equals 10/1.

Acetone Insoluble Antigen—The preparation and preliminary extraction of the heart muscle in the case of the acetone insoluble antigens, was the

TABLE I
TITRATION OF SIMPLE ALCOHOLIC ANTIGENS

ANTIGEN TAKEN C C	POSITIVE SLRUM		NORMAL SERUM	
	HEMOLYSIS		INHIBITION	
	ISOPROPYL ANTIOEN	ETHYL ANTIGEN	ISOPROPYL ANTIGEN	ETHYL ANTIGEN
0 000	Total	Total	None	None
0 004	None	75%	"	"
0 006	"	50%	"	"
0 010	"	None	"	"
0 020	"	"	"	"
0 040	"	"	"	"
0 060	"	"	"	"
0 080	"	"	"	"
0 100	"	"	"	"
0 140	"	"	60%	10%
0 160	"	"	Total	30%
0 200	"	"	"	Total

same as for the simple alcoholic extracts except that one liter of alcohol was used for each 100 gm of ground heart muscle

After extracting at 37° C for two weeks and filtering, the alcoholic extracts were evaporated at room temperature before a fan. The residues were then taken up with ether and the solutions transferred to stoppered bottles and left overnight for sedimentation.

The clear ethereal solutions were decanted, evaporated to a volume of 20 c c, and treated with 200 c c of acetone, each, for precipitation of the lipoids. The vessels were covered and allowed to stand overnight, in the ice box, to collect the precipitates. The acetone was decanted, and the precipitates were allowed to dry to the usual sticky consistency.

The yield of acetone insoluble lipoids obtained from 100 gm of heart muscle, extracted with 1000 c c of 91.1 per cent isopropyl alcohol, was 3.5 gm. From 100 gm of heart muscle 3.2 gm of lipoids, extracted with 1000 c c of 95 per cent ethyl alcohol were obtained.

Stock antigen solutions were prepared from these in the usual manner by dissolving 0.30 gm of lipoids in 10 c c of methyl alcohol and 1 c c of ether.

Titration was carried out in the same manner as for the simple alcoholic extracts with results as shown in Table II.

TABLE II
TITRATION OF ACETONE INSOLUBLE ANTIGENS

ANTIOEN TAKEN C C	POSITIVE SERUM		NORMAL SERUM	
	HEMOLYSIS		INHIBITION	
	ISOPROPYL ANTIGEN	ETHYL ANTIGEN	ISOPROPYL ANTIOEN	ETHYL ANTIOEN
0 000	Total	Total	None	None
0 004	None	None	"	"
0 006	"	"	"	"
0 010	"	"	"	"
0 020	"	"	"	"
0 040	"	"	"	"
0 060	"	"	"	"
0 080	"	"	"	"
0 100	"	"	"	"
0 140	"	"	"	"
0 160	"	"	"	Trace
0 200	"	"	"	10%

An examination of the results shown in Table II shows the antigenic powers of the two preparations to be identical. The antigen prepared with ethyl alcohol shows some inhibition of hemolysis when normal sera are used in amounts above 0.14 c.c., while the isopropyl alcohol product shows none in any of the amounts tested.

CONCLUSIONS

1 Isopropyl alcohol is superior to ethyl alcohol for the preparation of Wassermann antigens of the simple alcoholic type. It yields a product of superior antigenic power and shows relatively less anticomplementary action.

2 Isopropyl alcohol is superior to ethyl alcohol for the production of acetone insoluble antigens because it gives a more complete extraction of the acetone insoluble lipoids of normal tissues. The antigen produced is equal in antigenic power to that produced by use of ethyl alcohol and is somewhat superior as regards anticomplementary behavior with normal sera.

3 The use of isopropyl alcohol for this purpose is also to be recommended over the use of tax paid ethyl alcohol because it is less costly and is not subject to troublesome regulations.

SPIROCHETAL BRONCHITIS REPORT OF A CASE SUCCESSFULLY TREATED WITH ARSPHENAMINE

BY JOHN W. FISHER, M.A., M.D., TWIN FALLS, IDAHO

THE existence of pulmonary infections due to the spirochete of Vincent and to the fusiform bacillus has only recently been recognized. Credit is due to Pilot and Davis¹ for their pioneer work in this field, and those interested in this subject are referred to their exhaustive article for a complete discussion and bibliography. Within the last year several other papers have appeared on this subject, the most important of which is one by Kline and Berger.² Most of the cases reported have exhibited pulmonary abscesses and pulmonary gangrene. The following case is, therefore, of interest because of its gradual onset and because abscess formation did not occur.

REPORT OF CASE

Mrs. N. F. S., aged thirty-eight, developed acute bilateral pyosalpinx following the birth of her eighth child on September 21, 1924. A laparotomy was performed October 23, 1924, and both tubes and the left ovary were removed and drainage instituted. I saw her in consultation before the operation and learned that her previous health had been quite good. A careful physical examination at that time revealed no pulmonary pathology. There

Approved for publication by the Medical Director of the Veterans Bureau.
From the U. S. Veterans Hospital, Waukesha, Wisconsin.
Received for publication, February 2, 1926.

was moderately severe pyorrhea alveolaris. Ether was given and no immediate complications followed, but a bronchitis gradually developed and became progressively worse until January 20, 1925, when I saw her again in consultation. She had lost fifty pounds in weight, had a severe productive cough, and looked decidedly ill. There had been an irregular temperature, often higher in the morning, reaching 102° at times. Her pulse was quite rapid, varying between 100 and 110. The white count was 12,500, the red count was 3,500,000, and the hemoglobin was 65 per cent. Her breath and sputum were very offensive in odor. The latter was mucopurulent, very light brown in color, and contained small flecks of blood. Examination of the chest revealed many moist, moderately coarse râles scattered diffusely throughout both lungs, not more numerous in the apices. Breath sounds were roughened, but there were no evidences of large areas of consolidation. Radiograms showed mottling throughout both lungs, resembling that seen in influenzal pneumonia, but no abscesses. Repeated sputum examinations for tubercle bacilli were negative, but large numbers of fusiform bacilli and of Vincent's spirochetes, together with streptococci and pneumococci, were found. Three intravenous injections of nearsphenamine (0.15, 0.3, and 0.6 grams) were given in the course of two weeks with the result that the cough abated, the râles disappeared, the sputum became scanty and normal in character, and no more fusiform bacilli or spirochetes could be found. She looked and felt much better, and her appetite improved greatly. Two weeks later she developed a slight cough secondary to a rhinitis. A few spirilla were found in the sputum and an intramuscular injection of sulpharsphenamine was given to guard against a recurrence of the pulmonary infection. There has been no return of the bronchitis to date (December 1, 1925), and she writes that she never felt better. Her present weight is 128 lbs., a gain of 28 lbs. in ten months.

COMMENT

The similarity of this case to one of rapidly progressing pulmonary tuberculosis is apparent. In fact, this syndrome has actually been called pseudo-tuberculosis. Certain important differences, however, were noted. The temperature curve was not typical, being higher in the morning, and there were no night sweats. The classical findings of consolidation in the apices were absent, and the râles, which were somewhat coarser than those heard in tuberculosis, were scattered diffusely over the whole chest. The radiograph also was not typical as the apices were too clear, and the mottling was too general. In spite of these atypical findings, the diagnosis was in doubt until fusiform bacilli and spirochetes were noted while examining a smear for tubercle bacilli.

It was found that the spirochetes did not stain well with methylene blue, but the fusiform bacilli stained readily with this dye. The examiner should keep on the watch for the latter organism while searching for tubercle bacilli. The spirochetes can be readily demonstrated if a thin smear is stained deeply with carbolfuchsin and examined before decolorization. They were very numerous in this case before treatment was instituted. As a few organisms

are often present in saliva, the sputum should either be washed or care exercised to make the smear from the center of a mass of purulent sputum

Pyorrhea alveolaris has been mentioned by several authors as the source of the pulmonary infection and was present in this case. Proper oral hygiene is, obviously, an important prophylactic measure. Local treatment to the patient's gums was instituted promptly.

The rapid improvement in the bronchitis following neocarsphenamine administration was gratifying. Since pulmonary abscess and gangrene respond much less readily, it is important that an early diagnosis be made and that treatment be instituted promptly.

SUMMARY

A case of purulent bronchitis due to Vincent's spirochete and to the fusiform bacillus is reported in which prompt recovery followed the administration of neocarsphenamine intravenously. The importance and the simplicity of the early recognition of this infection are emphasized.

REFERENCES

- Plot, I., and Davis D. J. Studies in Fusiform Bacillus and Spirochetes in Pulmonary Abscesses, Gangrene, and Bronchiectasis. Arch. Int. Med. September 1924, **xxxiv**, 313.
Kline, B. S., and Berger S. S. Spirochetal Pulmonary Gangrene Treated with Arsphenamine. Jour. Am. Med. Assn. November 7 1920, **lxxxv**, No. 19, 1462.

REPORT OF A CASE OF COCCIDIOIDAL GRANULOMA WITH AUTOPSY FINDINGS*

By F. PIOLSCHEI, M.D., I. RIAN, M.D. AND A. P. KUEGER, A.B.
SAN JOSE, CAL.

SINCE Dickson's¹ review of the literature on coccidioidal granuloma, twelve new cases have been reported. The majority of the new cases (seven) were observed in California, the others were one from Missouri, one from Charleston, South Carolina, and one from Kansas. The latest case reported was from Chicago. This case came, however, originally from California.

We wish to report a new case which we observed in San Jose which ended fatally and was interesting in so far as we were able to obtain a pure culture of the fungus from the blood stream during life. This is the first case to our knowledge in which the organism could be demonstrated in the blood.

HISTORY

Mr. T. H., aged sixty one, was a cement and plaster contractor. His father died of apoplexy, one sister died of cancer of the stomach. Otherwise his family history was negative. When a small child he had an almost fatal infection following the opening of a boil and was many months in recovering. He had

*From the Laboratories of San Jose Hospital and Agnew State Hospital.
Received for publication January 26, 1926.

removal of the scab a rather sharply outlined ulcer was disclosed containing a thick gray-yellow mucoid pus. Its borders were not infiltrated and showed no signs of inflammatory reaction. On removing the pus a pale grayish-red, almost smooth ulcer remained, apparently involving only the corium.

On the frontal, left parietal, left and right temporal regions the integument was covered with dry-brownish-red scabs. Removal of the scabs brought to view partially healed operative incisions about 2 cm long. The borders of the incisions were slightly infiltrated, and on pressure a gray mucoid pus could be evacuated from all of them. The largest quantity of purulent material was obtained from the incision over the right temporal region. On palpating the underlying bony structures, distinct depressions could be noted.

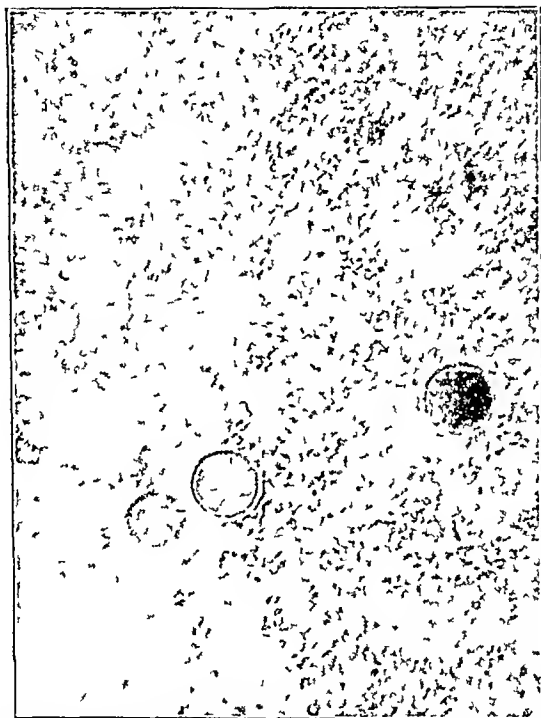


Fig 2—Typical spore-like microorganisms from pus of thumb. Drythiosin glycerin sodium acetate. Oil emersion Oc 4.

The skull cap showed the following lesions. Left upper third of coronal suture between marginal portion of frontal and parietal bones disclosed a subperiosteal and osteomyelitic abscess $20 \times 10 \times 6$ mm, forming an irregularly outlined ragged cavity extending in part beneath the external tabula into the frontal and parietal bones. The anterior part of left parietal bone, 2 cm above the squamous suture showed an abscess about 3 mm in diameter, cone shaped, extending into the diploe, not perforating through the internal tabula. The posterior part of the left parietal os 2 cm below the sagittal suture showed an abscess $10 \times 7 \times 2$ mm. The lower posterior part of parietal os showed a horse-shoe-shaped abscess $22 \times 10 \times 3$ mm perforating through the external tabula. The dura beneath was covered with granulation tissue. Two centimeters above the left squamous suture was a superficial ero-

sion of the external tabula 10×5 mm. The right upper portion of occipital suture near the lambda suture showed two subperiosteal abscesses, each with superficial erosion of external tabula $5 \times 5 \times 1$ mm and 5×2 mm. The left occipital suture disclosed an abscess $22 \times 12 \times 3$ mm with multiple punctiform perforations into the internal tabula. The lower posterior portion of left parietal bone showed just above the *incisura parietalis* a nonperforating abscess 12×9 mm.

Over the right medial malleolus there was an ulcer about 4×2 cm, its borders sharply outlined but without any infiltration. On pressure about 20 cc of slightly bloody, gray white pus was evacuated from the underlying structures. Enlargement of the abscess cavity showed that the subcutaneous



Fig 3—Section from lung showing several spore-like forms. Leltz Obj 4 and Oc 4

tissue had been transformed into a grayish yellow mucoid pus. The ligaments were surrounded with pus but not loosened. The periosteum of the medial malleolus was necrotic, the spongiosa was soft, friable, and infiltrated with pus, the articulation talocruralis was not involved and the cartilage was smooth and glistening.

Over the dorsum of the right foot there was a fluctuating area about the size of a dollar with the integument slightly raised and of a light smoky gray. In the center appeared a small opening which on pressure yielded a small quantity of gray yellow mucoid pus. Incision revealed a subcutaneous abscess. The underlying tendons of the long extensor muscles of the leg were bathed in pus. The second, third, and fourth *spatia interossea* were filled

with pus The short extensor muscles of the toes were necrotic and transformed into a smeary-gray-yellow material The periosteum of the second, third, and fourth metacarpal bones was lacking, the compacta soft and the spongiosa friable

Over the metacarpophalangeal joint of the right thumb there was a single incision about 2 cm long from which gray-yellow pus was obtained on pressure Enlargement of the opening showed the capsule of the joint to be covered with pus The surrounding muscles were pale, grayish-red and infiltrated with pus The periosteum of the distal end of the first metacarpal and of the proximal end of the first phalanx was necrotic, the compacta soft and the spongiosa firm The joint itself was not involved

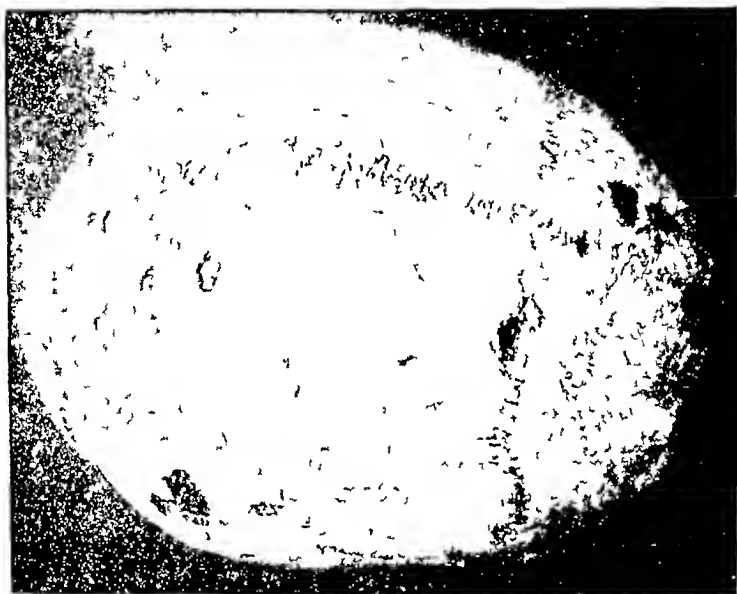


Fig 4—Macerated skull cap showing several osteomyelitic abscesses

Upon removal of the skin from the left supraclavicular region a creamy gray-yellow pus escaped from a small opening in the platysma which led into the supraclavicular fossa Upon enlarging the opening an abscess cavity was found extending upwards about 3 cm beneath the sternocleidomastoid muscle The periosteum of the acromial portion of the clavicle was lacking, the compacta roughened, and the surrounding structures showed no inflammatory reaction The cavity contained about 40 cc of pus, with no perforation into the apex of the pleural cavity The superficial and deep cervical, as well as the submaxillary lymph nodes were slightly enlarged, pinkish-gray, somewhat succulent, and on section were homogeneous pale gray-red

The subcutaneous adipose tissue had almost disappeared leaving only here and there a few islands of canary-yellow fatty substance Upon opening the pleural cavities, both lungs collapsed but were freely movable The large vessels and the pericardial sac were covered with some fat tissue The parietal and visceral pleura were smooth, moist and glistening There was no

fluid in the pleural cavities. The ductus thoracicus was without change. The heart was slightly enlarged, the myocardium flabby, easily torn, and of a pale, cloudy, grayish red color. The valves were without change.

The upper lobe of the left lung was fluffy, crepitating, slate gray and slightly anthracotic. The lower lobe was of a semisolid consistency, non crepitating, dark bluish gray somewhat anthracotic and on its anterior surface the visceral pleura was slightly thickened but smooth. On section the parenchyma of the upper lobe was gray red, and the cut surface finely granular with a great many miliary and submiliary grayish white nodules which were difficult to recognize being more numerous in the upper part. The parenchyma contained air throughout and on pressure yielded a large amount of frothy bloody fluid. The mucous membrane of the large bronchi was covered with tenacious gray mucus, and the small bronchi were filled with a glassy mucus. The bronchial mucosa was gray white smooth, and here and there slightly congested. There was no enlargement of the bronchial lymph nodes, and on section the cut surface was smooth and uniformly deep black.

The upper and middle lobes of the right lung were slate gray, somewhat anthracotic and slightly crepitating. The lower lobe was dark bluish violet and of a semisolid consistency, and upon palpation there was noted near the middle of the anterior surface just beneath the visceral pleura a firm nodule about the size of a pea. On section the parenchyma of the upper and middle lobes was slightly congested, gray red, its cut surface was noticeably granular and exhibited numerous gray white miliary and submiliary nodules. The parenchyma of the lower lobe was dark brownish red, edematous, and a bloody, frothy fluid dripped from the cut surface. Throughout the parenchyma were seen numerous gray white miliary nodules which on section proved to be gray yellow, dry, but without caseation. The large bronchi contained a quantity of gray, tenacious mucus, the small bronchi were also filled with a slightly bloody mucus. The mucous membrane was smooth, gray white, here and there congested. The bronchial lymph nodes were slightly enlarged, presenting on section a smooth, deep black, cut surface. One of the lymph nodes showed numerous miliary gray white nodules.

The pharynx, larynx, and esophagus did not show any lesions. The spleen was enlarged, fairly firm, bluish red, with slight capsular thickening. On section the pulp was gray red, follicles distinctly visible as grayish, opaque points and the trabeculae only visible here and there. Throughout the pulp was found many grayish yellow, opaque fairly well outlined nodules of a dry, cheesy consistency which measured from 1 to 3 cm. in diameter. Some of these nodules were surrounded by a small brownish red zone.

In the right kidney was found, irregularly distributed over the cortex many gray white, round, indefinitely outlined areas about the size of a pin head, which were not raised, but extended 2 to 3 mm. into the cortical substance.

After removal of the aorta and right iliac artery, there was found beneath the right psoas muscle an abscess about the size of a plum, containing a yellowish gray, thick, mucoid material. On removing the peritoneal cover

ing of the right side of the small pelvis and the promontorium, it was found that the abscess extended into the fifth and sixth lumbar vertebrae and into the intervertebral cartilages. The spongiosa of the anterior portion of both vertebrae was soft, pliable and could easily be removed with the knife. The whole spongiosa was bathed in a thick yellowish-gray pus.

The inguinal lymph nodes of the right side were enlarged, succulent, and on section pale gray-red.

The dura mater was thickened and showed over the right temporal area, corresponding with the above-described perforation of the right temporal



Fig. 5—Pure culture *Coccidioides immitis* from blood. Bouillon culture six weeks old.

bone, an area about 2 cm. in diameter, which was covered with a grayish red, smeary granulation tissue. The remainder of the external surface was smooth and glistening. Upon removing the brain about 50 cc. of a slightly bloody fluid dripped off.

The brain was slightly edematous and showed many bloody points which diffused into the surrounding brain tissue. The cortex was otherwise macroscopically without change. The lateral ventricles were filled with a slightly bloody fluid, the ependyma was smooth, the choroid plexus was pinkish-gray and distinctly edematous.

MICROSCOPIC FINDINGS

Abscess of the Foot (Eosin Hematoxylin Stain)

The epidermis was intact, but throughout the subcutaneous tissue were numerous more or less sharply outlined abscesses, and other places were diffusely infiltrated with leucocytes. Here and there were a few detached islands of epithelial cells. The leucocyte infiltrations were made up mainly of endothelial cells, polynuclear leucocytes, a few lymphocytes and plasma cells containing many cysts. Most of the cysts were surrounded by a double contoured membrane which stained either red or reddish blue and contained either well defined roundish bodies or honey comb like structures. Some of the cysts were phagocytized by large giant cells containing 15 to 20 nuclei or more, and some of the giant cells contained 2 or 3 spore containing cysts. The lymph vessels were distinctly visible, their lumens filled with mononuclear and polymorphonuclear leucocytes. The large vessels of the subcutaneous tissue showed a marked thickening of all three coats, the walls of some of the vessels were more or less infiltrated with large and small lymphocytes and plasma cells, the areolar fatty tissue was here and there infiltrated with mononuclear cells and lymphocytes.

Lung (Eosin Hematoxylin Stain)

Throughout the parenchyma there was numerous more or less well defined miliary and conglomerated inflammatory nodules. The majority of the areas originated in the peribronchial and perivascular connective tissue, extending into the adjacent alveoli. Others arose in the alveoli proper, being confined to one alveolus or to a group of alveoli. These areas were made up of collections of lymphocytes, lymphocytic plasma cells, endothelial leucocytes and polymorphonuclear leucocytes. The center of the nodules consisted of epithelioid cells with round or oblong nuclei and were surrounded by a small zone of homogeneous or granular protoplasm in which numerous fine fibrillae took origin. The periphery of the nodules was made up of dense masses of lymphocytes and lymphocytic plasma cells with here and there a few polynuclear leucocytes. Many of the nodules contained giant cells, the nuclei of which were either peripherally arranged or gathered near the poles. The centers of many nodules were necrotic consisting of a reddish stained granular debris and disintegrated nuclei. Spore bearing and nonspore bearing cysts in a double contoured capsule were present in the necrotic areas, some were phagocytized by giant cells. Some of the alveoli were filled with polynuclear leucocytes or with a homogeneous reddish stained exudate. The walls of the large and small blood vessels were thickened, especially the adventitia, and the connective tissue was hyaline degenerated. The perivascular lymph spaces were here and there densely filled with carbon pigment, the stroma of the septa was thickened but not involved in the inflammatory process.

Spleen (Eosin-Hematoxylin Stain)

The splenic pulp contained numerous abscesses, some being made up entirely of polynuclear leucocytes without cysts while others contained spore bearing or nonspore bearing cysts and one or two giant cells. Some nodules

showed a tubercle-like structure, made up of endothelial cells surrounded by lymphocytes and a few polynuclear leucocytes. They were of two types (necrosed or nonnecrosed), both showed a few cysts and giant cells



Fig 6—Pure culture *Coccioides immitis*
Agar

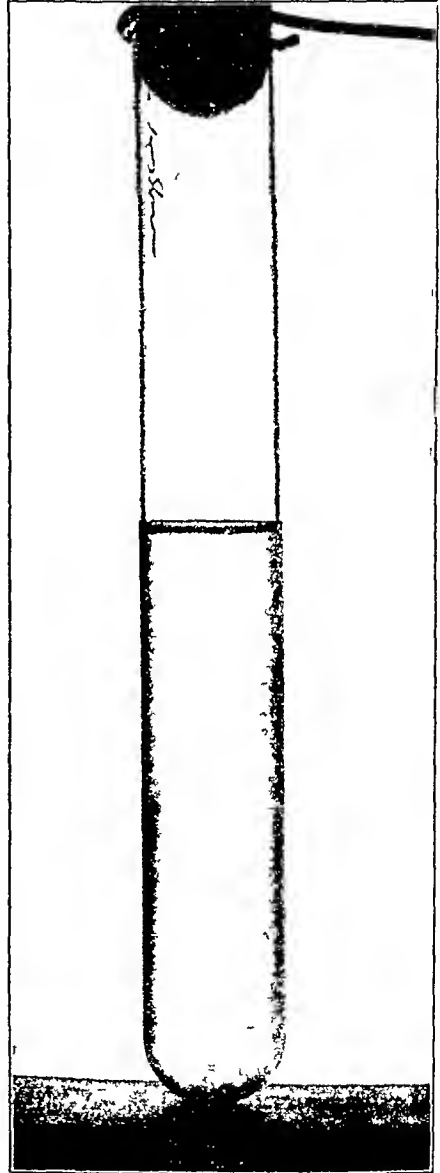


Fig 7—Pure culture *Coccioides immitis*
Bouillon.

Liver (Eosin-Hematoxylin and Sudan Stain)

The liver cells were extensively infiltrated with fine fat droplets, especially around the center of the acini. The remaining protoplasm was coarsely granular. The peripheral tissue showed marked round-celled infiltration and

in many places small tubercle like formations composed of epithelioid cells and lymphocytes. Some of the tubercles contained parasites, mostly adult forms.

Kidney (Eosin Hematoxylin Stain)

The capsule was slightly thickened and was here and there infiltrated with round cells. Throughout the cortex were many aggregations of round cells and leucocytes. The capillaries of the cortex as well as the glomeruli, were markedly engorged with blood. The cytoplasm of the endothelial cells was distinctly vacuolated, while the nuclei were pyknotic. The capsular space was filled with a hyaline material and a few vacuolated endothelial cells. The lumen of the ascending tubules contained a fine circular reticulum.

The walls of the larger blood vessels were markedly thickened especially the media. The adventitia was more or less sclerosed the endothelium lining unchanged. The mucous membrane of the pelvis showed occasional infiltrations with round cells and leucocytes.

Myocardium (Sudan Hematoxylin and Eosin Hematoxylin Stain)

The majority of the muscle fibers contained minute fat droplets. The striations of the fibers were indistinct the nuclei pyknotic. The interstitial tissue was markedly increased and contained large fat globules. The walls of the large and medium sized blood vessels were greatly thickened.

Suprarenal Capsule (Sudan Hematoxylin Stain)

The lipid content of the cells of the fascicular zone was considerably diminished and consisted of very small fat droplets.

EXPERIMENTAL INOCULATION

Inoculation with the original pus or with a few days old culture of the fungus in guinea pigs, white mice, and white rats showed the guinea pig to be the most susceptible. The original pus was more virulent than the cultures, since all the guinea pigs inoculated with it died, of the guinea pigs inoculated with the pure culture of the fungus only 70 per cent succumbed. White rats were retractive.

Successive inoculation from guinea pig to guinea pig increased the virulence of the fungus. Intraperitoneal inoculation killed guinea pigs in about four weeks, while subcutaneous inoculation killed them in from two to three months. If spore bearing mycelia were injected subcutaneously, the mycelia and spores disappeared in a few days, and an abscess developed containing the typical spore bearing cysts. From the pus of this abscess a pure culture of the fungus could be obtained.

Intraperitoneally inoculated animals showed a large caseous mass adherent to the peritoneum at the site of injection. The intestines were covered with a slimy mucoid exudate. The liver was covered with numerous small grayish white to porcelain white flat nodules. The lungs and spleen showed small gray white nodules, and in both pleural cavities was a serous fibrinous exudate. The inguinal lymph nodes were enlarged, edematous homogeneous,

pinkish-gray, and contained a cheesy material not unlike tubercular cheese. The testicles were enlarged and infiltrated with a thick yellowish cheesy material. From all these lesions, it was possible to obtain a pure culture of the fungus. Upon sectioning the various organs, typical cysts were found.

CULTURAL FINDINGS

Cultures made from the various abscesses on plain agar incubated at 37° C for three to four days, yielded pure colonies of a fungus-like growth, not unlike a culture of tubercle bacilli. The colonies were first round and discrete, then became more profuse in a few days, and eventually covered the entire surface of the medium.

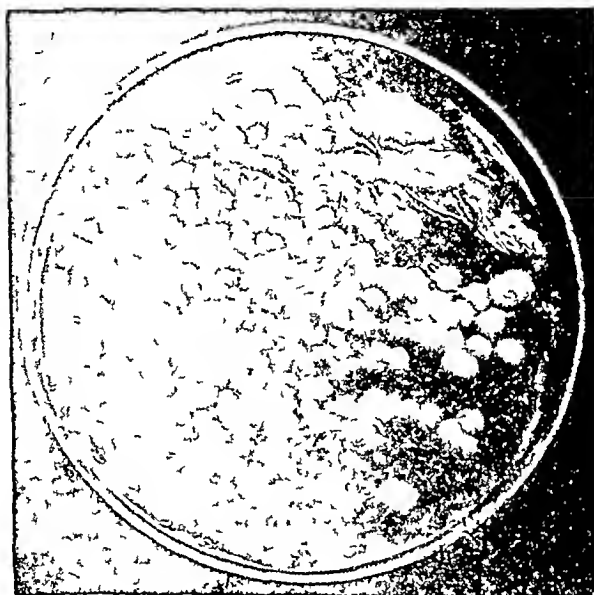


Fig. 8—Six-day agar plate showing numerous discrete colonies

On agar plates, containing only a few colonies, large colonies developed which measured three to four cm in diameter and showed concentric rings not unlike Lisegang's diffusion rings. The colonies were difficult to remove from the surface of the agar because of the deep growing hyphae.

At room temperature the development of the colonies was slower, requiring six to eight days, but the aerial hyphae seemed to develop more abundantly than at 37° C. Equally good growth was observed on agar to which various sugars were added (lactose, maltose, sucrose, arabinose, L-Xylose, D-Galactose, and glucose). The most luxurious growth took place on sucrose agar.

In plain bouillon, a white flocculent growth at the bottom of the tube was noted. The supernatant liquid remained perfectly clear. Bouillon cultures three to four weeks old showed a slight brownish tinge, and in some there appeared a thick wrinkled scum on the surface. Bromcresol-purple bouillon to which the above-mentioned sugars were added showed fermenta-

tion of the sugar in each case. No gas formation was noted, while there were various degrees of acid production. Maltose and sucrose bouillon gradually turned alkaline.

Bromeresol purple milk showed no evidence of change during the first three or four days. After six or seven days the milk turned acid and became peptonized, and at a later date it became alkaline. A light greenish scum gradually formed on the surface.

Gelatine cultures developed a surface growth similar to that occurring on agar, but was not as abundant as on the latter medium and the formation of hyphae was less pronounced. Liquefaction of the gelatine gradually occurred.

Plain or glycerinated potatoes yielded a heavy white growth with abundant aerial hyphae. No growth occurred on native decoctions of plums, raisins, or apricots.

In Fraenkel's Synthetic Medium a good growth similar to that in bouillon, was obtained. The fungus is aerobic; no growth taking place under strict anaerobic conditions. If the cultures were protected from drying growth continued for several months and the cultures remained alive for six months.

Hanging drops prepared from bouillon cultures showed septated mycelia with true dichotomic branching. No spores developed in the mycelia as long as they were in the liquid medium. The aerial hyphae developed club shaped spores as described by Ophuls. If the spores were seeded in bouillon or on agar they formed new hyphae. Potato cultures yielded an abundance of spores.

The organism was easily stained with basic aniline dyes and was gram positive and nonacid fast.

SUMMARY

The case reported by us presents a typical infection with *Coccidioides immitis* of about six months' duration and with fatal termination. The initial clinical symptoms were a bronchitis with slight fever, general malaise and debility. After the temperature had subsided pain developed in the left hip, right ankle, and thumb simulating articular rheumatism. An abscess developed on the right side of the nose followed by multiple abscesses of the head, thumb, and right malleolus. The abscesses were all subperiosteal, did not heal, and did not respond to any treatment. From the character and the distribution of the lesions a blood stream infection was suspected and was confirmed by a positive blood culture obtained shortly before death. The immediate cause of death was probably a toxemia.

Autopsy revealed multiple subperiosteal and osteomyelitic abscesses of the skull cap, of the second, third and fourth metatarsal bones of the right foot, an abscess of the psoas muscle and suppurative osteomyelitis of the fourth and fifth lumbar vertebrae, subperiosteal abscess of the left clavicle extending beneath the sternocleidomastoid muscle. Miliary pneumonumycosis of both lungs, chronic bronchitis, fatty degeneration of the myocardium and chronic fibrous myocarditis, localized external suppurative pachymeningitis over the right temporal area, slight edema and hyperemia of the brain.

respond to the peroxidase test that colors the "transitional" leucocytes in blood smears of the same individual. The result of this simple test alone is sufficient basis for the unqualified statement that such phagocytes are not monocytes ("transitional" leucocytes). The peroxidase reaction has been subjected to criticism because the methods of using the various chemicals which have been employed to elicit it gave different results on the same tissue due to variations in the solutions used for the reaction or to the method of fixation of the tissue. However, if the reagent is applied by the same method to a given tissue the results are the same and it is in this sense that the test is significant. I² found that when paraffin sections of the various tissues and organs were treated with benzidin the peroxidase-reacting cells were found only in the bone-marrow, spleen, and within the blood vessels. Foci of reacting cytoplasm were observed in some of the endothelial cells lining the sinusoids of the liver, but these had the character of phagocytized material. The reticuloendothelium of the lymph nodes and many of the free cells within the sinuses did not react. Subsequently smears were made of human lymph nodes and these side by side with smears of human blood were treated with benzidin by the same technic. The lymph node is almost entirely devoid of reacting cells.³ The "transitional" leucocyte, or monocyte, of human blood quite certainly arises from the bone-marrow and perhaps to some extent from the spleen although there appears to be no decrease in the number of this leucocyte after splenectomy.⁴ The relationship between "transitional" and polymorphonuclear leucocytes with neutrophilic, eosinophilic, or basophilic granules has not been fully determined. Naegeli states that the monocyte arises from a younger cell, the monoblast, in the marrow. Although the cell has neutrophilic granules the granulation is apt to be less pronounced than that of either the neutrophilic myelocyte or the older polynuclear leucocyte. On the other hand, in the bone-marrow and in the blood of individuals with chronic myeloid leucemia there are neutrophilic myelocytes with relatively few granules and the monocyte may ultimately prove to be an older form of such myelocytes and quite closely related to the polymorphonuclear neutrophile. Not infrequently increases in "transitionals" have been observed during polynuclear leucocytoses and it should be kept constantly in mind that the "transitional" cell, or monocyte is a myeloid cell. The peroxidase* method of staining is required for the accurate identification of monocytes. Otherwise the scant number of granules present in some of these cells after polychrome staining may be overlooked, or, when present may even be confused with the azurophilic granules. In peroxidase preparations the only possibility of error is in mistaking monocytes for immature polynuclear neutrophiles. The cyto

*A method (Jour. Am. Med. Assn. 1920 lxxiv 17) employing benzidin for the peroxidase reaction has been in use for more than six years. It is simple and satisfactory. Smears of blood exudates or tissues allowed to dry in the air for a half hour not for more than twenty-four hours are covered for thirty seconds with the benzidin solution which consists of 100 mg. of benzidin (dry powder) dissolved in 25 c.c. of 80 per cent pure methyl alcohol to which 1 or 2 drops of hydrogen peroxide has been added. At the end of the half minute the alcoholic solution is diluted with an equal quantity of distilled water. The diluted reagent usually colors the granules an intense brown within three minutes. The reagent is washed off with water and the preparation blotted with blotter paper. It is now ready for the counterstain which is made by placing Wright's stain on the preparation and at once diluting it with water. The diluted stain is allowed to act for about five minutes since the nuclei stain less readily after the treatment with benzidin. Hematoxylin (Harris with acetic acid) diluted 1:5 applied for one minute followed by washing and staining with eosin (0.1 per cent aqueous solution) for one minute is an excellent counterstain. The benzidin solution keeps for several months.

plasm of the latter is usually heavily studded with neutrophilic granules and the nucleus is more pyknotic

"Large Mononuclear" Leucocytes (Lymphendotheliocyte) — Consideration of the nonperoxidase reacting mononuclear phagocyte of the blood is less simple. The possibility of the 'transitional' leucocyte or monocyte, being the sole mononuclear phagocyte of normal blood was considered, but I found⁵ that a certain number of the mononuclear cells which do not react to benzidin ingest carbon when brought into contact with it at incubator temperature. There is therefore a nonperoxidase mononuclear cell that is phagocytic. These cells are less numerous than the monocytes and for their demonstration it is essential to collect the leucocyte layer of the citrated blood. During incubation with carbon the outlines of the ameboid cells assume a very irregular form but in size the nonperoxidase reacting phagocyte corresponds to the 'large mononuclear' leucocyte. The combination of the peroxidase test with the phagocytic method⁵ is the best one available for the positive identification of the lymphendotheliocyte ("large mononuclear" of the blood). A simpler technique is desirable.

The evidence indicates that this phagocyte is derived from the lymphoid reticuloendothelium. Sabin, Doan, and Cunningham⁶ brought these cells into contact with dilute neutral red and found that a focus of dye granules (rosette) appeared in the cytoplasm at one side of the nucleus. These investigators⁷ concluded that this rosette cell was derived from reticular tissue. With this I agree,⁸ but in my experiments the rosette cells were found to be most numerous in the lymph nodes. Cunningham, Sabin and Doan⁷ were unable to demonstrate any cells of this type in mesenteric lymph nodes. I do not, therefore, hold the same view as these investigators in regard to the distribution of the reticular tissue that gives rise to the rosette cells. Also it is essential to recognize two types of reticular tissue since the endothelium of blood capillaries may grow as reticular cells but it does not give rise to the rosette form of phagocyte.⁸ Sabin and coworkers, Cunningham and Doan, in common with a great many others speak of the rosette cells as monocytes and evidently regard them as identical with the Naegeli monocytes. The Naegeli monocyte of myeloid origin has not been demonstrated in the blood of rabbits and is present in scant numbers in the blood of normal guinea pigs. In the human being where the rosette cell (lymphendotheliocyte) is seen so clearly in tuberculous tissue and in normal lymph nodes, it may be demonstrated emphatically by peroxidase staining that the rosette phagocytes are not the Naegeli monocytes and there is no evidence that the two types of cells are at all related.

Since the identification of the rosette cell, or lymphendotheliocyte is dependent upon the contact of the living cell and dilute neutral red solution it is not easy to determine the complete distribution of the reticular tissue that gives rise to this type of phagocyte. By mordanting the tissue in Zenker formal solution after injection of the dye into lymph nodes it was possible to see foci of dye granules in the larger reticular cells in the medulla of the nodes.^{8, 9} Tissue cultures proved to be an effective method for bringing dilute solutions of neutral red into contact with the individual cells of the cultures. The reticuloendothelial cell (rosette) of the lymphoid tissue was found to be

almost the sole phagocyte of the lymph node cultures³ In tissue cultures of the spleen⁶ the rosette type of phagocyte predominates and the lymphoid type of reticuloendothelium is therefore thought to be present in the spleen as a fixed tissue The spleen is evidently only one of the contributing sources for this phagocyte, since splenectomy⁴ causes no demonstrable decrease in the number of these cells appearing in experimental exudates It has constantly been observed that this cell readily proliferates, not only in tissue cultures but also in the exudates and in various tissues where it occurs However, it is very probable that the lymph nodes and the spleen, where these cells are present as a fixed tissue, constitute the chief source of lymphendothelocyte supply when the demand for these leucocytes is made In the routine examination of the lymph glands one is impressed by the great variation in the number and size of the reticular cells of the sinuses It seems likely that the number of cells of the rosette type appearing in the blood will be found to vary with the activity of lymphoid reticuloendothelium in the lymph nodes and elsewhere

Hemendothelocyte—This is the phagocyte derived from the blood vascular endothelium The lymphoid reticuloendothelium is only one of the two types of reticular tissue It is for this reason that it is no longer sufficient to speak of endothelial leucocytes without designating their source There are two varieties of endothelial leucocytes In tissue cultures of the liver of rabbits the hemendothelocyte grows in reticular form with cytoplasmic branches connecting the individual phagocytes When supravitality stained with neutral red it is a diffuse granule-hyaline type of cell That is, the dye granules are scattered about diffusely in the cytoplasm Often the dye is scant or is entirely absent with the cell appearing as an unstained hyaline cell^{3, 4, 5} This cell may appear in the peripheral blood of rabbits after the injection of large quantities of India ink⁸ There is no evidence that it is present in the normal human blood It is almost the sole phagocyte of tissue cultures of rabbit liver³ In tissue cultures of rabbit spleen it is constantly present but is much less numerous than the rosette cell⁶

Discussion—Although the relative percentages of the granular leucocytes recorded by hematologists are about the same, one has only to consult a dozen of the current textbooks to find expression of a wide variation as regards the mononuclear leucocytes Some workers recognize only a single group, usually either "large mononuclear" or "transitional," while others divide the leucocytes into the two groups Within the groups, whether one or two, there are found to be variations of several hundred per cent in the figures given as the mean, minimum, and maximum for normal individuals The explanation of this lack of standard for the mononuclear cells is that the technical methods now in common use in differential blood counting do not enable one to identify these cells accurately The peroxidase-reacting monocyte of Naegeli, the "transitional" leucocyte, is the most numerous of the mononuclears To determine accurately the number in human blood, the smears should be stained by a simple peroxidase method that colors only neutrophilic and eosinophilic granules By such an examination it should be possible to establish the normal percentage for this form of myeloid cell, and finally to determine the nature and occurrence of true monocytoses

That there is present in normal human blood a second nonlymphocytic mononuclear cell is subject to direct proof. In general structure it corresponds to the "large mononuclear." It is a nonperoxidase reacting cell that acquires neutral red in the form of the rosette of Sabin, Doan, and Cunningham by the method of supravital staining. The evidence warrants the emphatic statement that this leucocyte is derived from reticuloendothelium of the type found in lymph nodes. Further work is required to determine fully the distribution of this type of reticular tissue. The readiness with which this cell undergoes mitosis also indicates that these leucocytes may arise in the tissues wherever they happen to be, provided they receive the proper stimulus. To identify the lymphendothelocyte in peroxidase preparations it is necessary only to differentiate it from the larger lymphocytes. However, a method simpler than that of supravital staining, which would mark this type in a positive way, would prove very useful.

By the experimental stimulation of the blood vascular endothelium, leucocytes of hemendothelial origin can be made to appear in the peripheral blood.⁸ There is no evidence that this form of leucocyte is present in normal human blood. It is a phagocyte that has comparatively little affinity for neutral red. If neutral red granules do appear in its cytoplasm they have a diffuse distribution.

CONCLUSIONS

1 The "transitional" leucocytes (monocyte of Naegeli) is the peroxidase reacting mononuclear phagocyte of human blood. It is one of the granular leucocytes of myeloid origin. When applied as a chemical test by a prescribed technique the peroxidase reaction permits the accurate differentiation of this cell from the nonperoxidase reacting phagocytes that have incorrectly been called monocytes.

2 The second type of mononuclear phagocyte present in the normal peripheral blood is characterized by a focus of dye granules when the living cell is brought into contact with a dilute solution of neutral red. It is a nonperoxidase reacting cell which corresponds in its general structure to the leucocytes that have commonly been designated as "large mononuclears."

3 Under experimental conditions the third type of mononuclear phagocyte which is derived from the blood vascular endothelium may appear in the peripheral blood. By the method of supravital staining it may present a diffuse granulation or may be devoid of granules.

REFERENCES

- ¹Naegeli, O. Das Blut, Aschoff's pathologische Anatomie 1923, n. 157.
- ²McJunkin, F. A. Peroxidase Staining with Benzidin in Paraffin Sections of Human Tissues. Anat. Record, 1922, xxiv, 67.
- ³McJunkin, F. A. Supravital Staining of Cultures of Lymph Nodes and Liver Endothelium. A paper read before the American Association of Pathologists and Bacteriologists, April 3, 1926, at Albany, N. Y., Archiv für Experimentelle Zellforschung, 1926, iii, 166.
- ⁴McJunkin, F. A. The Origin of the Mononuclear Phagocytes of the Peritoneal Exudates, Am. Jour. Path., 1925, i, 305.
- ⁵McJunkin, F. A. The Identification of Three Types of Mononuclear Phagocytes in the Peripheral Blood, Arch. Int. Med. 1925, xxxvi, 799.

- *Sabín, F R, Doan, C A, and Cunningham, R S The Separation of the Phagocytic Cells of the Peritoneal Exudate into Two Distinct Types, *Proc Soc Exper Biol and Med*, 1924, *xxi*, 330
- *Cunningham, R S, Sabín, F R, and Doan, C H The Development of Leucocytes, Lymphocytes, and Monocytes from a Specific Stem Cell in Adult Tissues, *Contributions to Embryology*, 1925, *xxi*, No 84, 227 Carnegie Institute of Washington
- *McJunkin, F A Identification of Two Types of Mononuclear Phagocytes in the Peripheral Blood of Rabbits, *Proc Soc Exper Biol and Med*, 1925, *xxiii*, 64
- *McJunkin, F A The Rosette and the Diffuse Granule Hyaline Types of Phagocytes in Tissue Cultures of Rabbit Spleen Stained Supravivally with Neutral Red, *Proc Soc Exper Biol and Med*, May, 1926 In print

A SIMPLE METHOD FOR THE DETERMINATION OF CALCIUM IN WHOLE BLOOD*

By W R CAVEN, M B (TOR), AND A CANTAROW, M D, PHILADELPHIA, PA

IN THE course of our studies in calcium metabolism¹ - it became necessary to make determinations of calcium in whole blood as well as in serum. The following method was devised, based upon the Clark-Collip modification of the Kramer-Tisdall method for serum calcium.

Principle—The calcium is precipitated as calcium oxalate, the blood being hemolyzed by the addition of distilled water. The calcium oxalate is transformed by sulphuric acid into oxalic acid which is titrated with potassium permanganate.

Method—Two c.c. of 4 per cent ammonium oxalate are put into an accurately graduated centrifuge tube. To this approximately 2 c.c. of blood are added, the exact amount being noted. Distilled water is immediately run in to the 15 c.c. mark. The tube is inverted a few times until the contents are thoroughly mixed. The mixture is allowed to stand for one hour and is then centrifuged at high speed for ten minutes. The supernatant fluid is poured off and the tube inverted in a rack for five minutes, the mouth of the tube resting on a pad of filter paper. The precipitate is washed once with 5 c.c. of distilled water and once with 3 c.c. of dilute ammonium hydroxide (2 c.c. of concentrated ammonium hydroxide and 98 c.c. of distilled water), centrifuging and draining each time as before described. Then 2 c.c. of approximately normal H_2SO_4 are added, being blown directly upon the precipitate to break up the mat. The tube is placed in a boiling water bath for about one minute and the oxalic acid is titrated with 0.01 normal potassium permanganate in a water-bath at 75° C.

Calculation—The titration value multiplied by 10, if the permanganate is exactly 0.01 normal, gives the amount of calcium in milligrams per 100 c.c. of whole blood, if the amount of blood used was 2 c.c.

Following hemolysis and centrifugation a small precipitate is thrown down with the precipitate of calcium oxalate, which we believe to consist of the stroma of the disrupted red cells. This in no way interferes with the

*From the Department for Diseases of the Chest, Jefferson Hospital, Philadelphia.
Received for publication May 24 1926

reaction or titration. Varying amounts of added calcium have been successfully recovered, and the experimental error is no greater than in the original test as applied to the determination of serum calcium.

Using this method we have found the normal range of whole blood calcium to be from 6.5 to 9.5 mg. per 100 c.c. the results obtained being consistent

REFERENCES

- ¹Gordon, B., Lawrie, A. K. and Rourke, J. I. The Effect of Parathyroid Hormone on Certain Signs and Symptoms in Pulmonary Tuberculosis. A Preliminary Report (Accepted for publication by the Jour. Am. Med. Assn.)
- Cantarrow, A., Caven, W. R. and Corliss, R. Some Changes in the Chemical and Physical Characteristics of the Blood Following the Administration of the Parathyroid Hormone with Special Reference to the Clotting of the Blood (Accepted for publication by the Arch. Int. Med.)
- ²Clark, F. P., and Collip, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with a Suggested Modification Jour. Biol. Chem. March 1925, LXIII, No. 2

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LeSandier and Verge White of Egg Culture Medium for the Gonococcus *Compt rend Soc de Biol*, 1925, *xxii*, 227

Carefully separate the white of egg from the yolk and to one part of egg white add three parts of distilled water. Shake vigorously in a closed flask containing glass beads until the mixture becomes a homogeneous emulsion. Filter through glass wool and add 6 cc of glycerin to each 100 cc of filtrate. Sterilize for 30 minutes at 115° C. The resultant product is a viscous, slightly opalescent emulsion. One part of this emulsion is added aseptically to two parts of nutrient agar and slants prepared. On this medium the gonococcus colonies are similar to those seen on ascitic agar. All the characteristics of the organism are preserved. On this medium successful transplants have been made after 115 hours.

Burke, V., and Newton, J. L. Preparation of Gentian Violet Solutions for Intravenous Injection *Jour Am Med Assn*, Feb 20, 1926, *lxxxvi*, 529

In the preparation of dye solutions for intravenous therapy the following factors must be taken into consideration: toxicity of the dye for body cells, bactericidal action in the presence of body fluids, especially blood, reaction of solution, osmotic pressure, stability and solubility of the dye in the solvent.

The authors report their studies of the most suitable solutions of gentian violet for intravenous injection and suggest that some of the uncertainty regarding the therapeutic value of gentian violet solutions may be due to variations in the solutions, as well as to variations in the defensive mechanism of the host.

The reaction of gentian violet solutions should be kept as near neutrality as possible, the more alkaline the solution the less the toxicity and the greater the bactericidal activity. A stable alkaline solution cannot, however, be prepared.

The choice of a solvent for gentian violet lies between a 3 per cent sodium bicarbonate solution and a buffered solvent.

The bicarbonate should be added after the dye is in solution and, as the solution decomposes rapidly, it must be freshly prepared and injected immediately.

A solution of 0.3 gm of potassium dihydrogen phosphate and 0.387 gm of dipotassium hydrogen phosphate in 100 cc of distilled water is a very satisfactory solvent.

The reaction is near neutrality and the solution comparatively stable.

The maximum dose of the dye will vary with the solvent and, as the toxic action is cumulative, should not be repeated too frequently.

Walker, J. E. Effect of Mercurochrome—220 Soluble on the Germicidal Properties of Fresh Defibrinated Blood *Arch Path and Lab Med*, February, 1926, *i*, No 2, p 200

Three cc of freshly drawn defibrinated human blood were placed in each of nine test tubes. To each tube, except the ninth which was the control and received 0.1 cc of normal saline, was added 0.1 cc of a solution of mercurochrome of such strength as to give the desired concentration in the total volume.

After mixing, 0.1 cc of a bacterial suspension was added. A tenth tube containing 3 cc of normal saline also received 0.1 cc of bacterial suspension.

This tube was plated at once to determine the number of organisms present and the other tubes were plated after incubation at 37° C for varying periods (2 to 24 hours).

Mercurochrome in concentrations of 1:25,600 to 1:400 had no appreciable effect on the bactericidal activity of fresh defibrinated blood on colon bacilli, 1:200 mercurochrome destroys this activity.

Staphylococci and streptococci grow much more luxuriantly in blood containing mercurochrome 1:400 than in blood without mercurochrome probably because of the injurious action of the dye on the leucocytes.

The use of mercurochrome in septicemia is empirical. Any beneficial results are not due to a specific action on the causative bacteria and if favorable clinical reports continue to be received, their mechanism remains a problem to be worked out.

John, H. J. Preservation and Transportation of Blood for Chemical Study. Arch. Path. and Lab. Med., February 1926, No. 2, p. 248.

Twenty mg. of a 10:1 mixture of finely powdered sodium fluoride and thymol will satisfactorily preserve 10 cc. of blood for chemical analysis for five to seven days. As much as 150 mg. of this mixture does not alter the blood sugar.

Bunting, C. H. and Howells, E. Leucocytic Reactions in Smallpox, Chickenpox, Scarlet Fever, Measles and Mumps. Arch. Path. and Lab. Med. February 1926, No. 2, 189.

Daily total and differential leucocyte counts were made in the diseases noted above. The patients were all young adults of university age, counts were all made at the same time of day (early morning) in each case.

The following classification of leucocytes was employed: neutrophils, eosinophils, basophils, small lymphocytes, large mononuclears and transitionals.

The leucocytic pictures seen were as follows:

Scarlet Fever. A maximum neutrophilic leucocytosis occurred on the day of the appearance of the rash, followed by a steady but gradual diminution of the total and neutrophilic count during the course of the disease.

Eosinophils show a rather sharp rise both in percentage and number. Basophils are uninfluenced. The lymphocytes show an early sharp percentage fall, followed by a gradual recovery reaching a peak about the end of the first week. The monocyte curve follows the lymphocyte curve.

The neutrophil curve is interpreted as a reaction to living organisms (streptococci) and the lymphocyte curve as a reaction to toxins.

Smallpox. Moderate leucopenia usually three days before the appearance of the eruption and persisting for two to four days afterwards.

A well marked leucocytosis then occurs.

There is an early relative and absolute increase in neutrophils followed by a rapid drop below normal. When the leucocytosis develops the neutrophilic percentage remains low but the total number remains near normal.

Eosinophils and basophils show no striking change. An early reduction in the lymphocytes is followed by a sharp rise with great variation in the size of the cells.

In early cases certain bodies—classified as Councilman bodies—were seen in the large lymphocytes. One type was protoplasmic about the size of the nucleus staining a light clear blue with Wright's stain; others seemed to be small reddish (metachromatic) granules often surrounded by an apparent vacuole and seen in the nuclei. They are believed to be peculiar to the disease.

Chickenpox. Qualitatively the picture follows that of smallpox, quantitatively the range of cellular variation is smaller.

Measles. Primary leucopenia with a percentage rise in neutrophils followed by a rapid decrease. The basophils show no marked change but there is a tendency toward moderate eosinophilia. There is an initial lymphopenia influenced by the intensity of the infection.

MYCOLOGIC FORMULAS

Glucose

Monilia balcanica (Cast) ----- + = Glucose

Levulose

Monilia krusei (Cast) ----- + }
Monilia balcanica (Cast) ----- 0 } = Levulose

Maltose

Monilia tropicalis (Cast) ----- + }
Monilia macedoniensis (*Monilia macedoniensoides*), (Cast) ----- 0 } = Maltose
Monilia metalondinensis (Cast) ----- + }
Monilia macedoniensis (Cast) or (*Monilia macedoniensoides*) ----- 0 } = Maltose
Monilia pinoyi (Cast) ----- + }
Monilia krusei (Cast) ----- 0 } = Maltose
Monilia pinoyi (Cast) ----- + }
Monilia macedoniensis (*Monilia macedoniensoides*), (Cast) ----- 0 } = Maltose
Monilia tropicalis (Cast) ----- + }
Monilia rhoi (Cast) ----- 0 } = Maltose
Monilia pinoyi (Cast) ----- + }
Bacillus morgani (Cast and Chalm) ----- 0 } = Maltose
Monilia metalondinensis (Cast) ----- + }
Bacillus morgani (Cast and Chalm) ----- 0 } = Maltose

Galactose

Monilia metalondinensis (Cast) ----- + }
Monilia pinoyi (Cast) ----- 0 } = Galactose
Monilia metalondinensis (Cast) ----- + }
Monilia krusei (Cast) ----- 0 } = Galactose
Monilia macedoniensis (Cast) ----- + }
Monilia tropicalis (Cast) ----- + } = Galactose
Monilia bronchialis (Cast) ----- 0 }
Monilia tropicalis (Cast) ----- + }
Monilia macedoniensis (Cast) ----- + } = Galactose
Monilia krusei (Cast) ----- 0 }
Bacillus paratyphosus B (Schott) ----- + }
Bacillus morgani (Cast and Chal) ----- + } = Galactose
Monilia krusei (Cast) ----- 0 }

Saccharose

Monilia tropicalis (Cast) ----- + }
Monilia metalondinensis (Cast) ----- 0 } = Saccharose
Monilia tropicalis (Cast) ----- + }
Bacillus coli communis, sensu stricto (does not ferment saccharose),
(Escher) ----- 0 } = Saccharose
Monilia tropicalis (Cast) ----- + }
Bacillus paratyphosus B (Schott) ----- 0 } = Saccharose
Monilia macedoniensis (Cast) ----- + }
Bacillus coli communis, sensu stricto (Escher) ----- 0 } = Saccharose
Bacillus coli communis ----- + }
Monilia macedoniensis (Cast) ----- + }
Bacillus paratyphosus B (Schott) ----- 0 } = Saccharose
Bacillus coli communis ----- + }
Bacillus coli communis, sensu stricto (Escher) ----- 0 } = Saccharose
Bacillus neapolitanus (Emmerich) ----- + }
Bacillus coli communis, sensu stricto (Escher) ----- 0 } = Saccharose
Bacillus asiaticus (Cast) ----- + }

Inulin

<i>Monilia macedoniensis</i> (<i>Monilia macedoniensoides</i>) (Cast) -----	+	}	= Inulin
<i>Monilia tropicalis</i> (Cast) -----	0		
<i>Monilia macedoniensis</i> (Cast), (<i>Monilia macedoniensoides</i>) -----	+	}	= Inulin
<i>Monilia rhoi</i> (Cast) -----	0		
<i>Monilia macedoniensis</i> (<i>Monilia macedoniensoides</i>), (Cast) -----	+	}	= Inulin
<i>Bacillus coli communior</i> (Escher) <i>Bacillus pseudocoli</i> (Cast),			
<i>Bacillus neapolitanus</i> (Emm) -----	0		
<i>Monilia macedoniensis</i> (<i>Monilia macedoniensoides</i>), (Cast) -----	+	}	= Inulin
<i>Bacillus asiaticus</i> (Cast) -----	0		

Inositol

<i>Bacillus paratyphosus</i> B, Var M (Schott) -----	+	}	= Inositol *
<i>Bacillus paratyphosus</i> A (Schott) -----	0		

Glycerol

<i>Bacillus asiaticus</i> (Cast) -----	+	}	= Glycerol *
<i>Bacillus paratyphosus</i> B, Var M (Schott) -----	0		
<i>Bacillus columbensis</i> Strain L (Cast) -----	+		

CHEMICONICOLOGIC FORMULAS

Saccharose

Fehling -----	0	}	= Saccharose
<i>Monilia tropicalis</i> (Cast) -----	+		

Lactose

Fehling -----	+	}	= Lactose
<i>Bacillus paratyphosus</i> B (Schott) -----	00		
<i>Bacillus coli communis</i> (Esch) -----	+		

Pentose

Fehling -----	+	}	= Pentose *
<i>Monilia tropicalis</i> (Cast) -----	0		
<i>Bacillus paratyphosus</i> B (Schott) -----	+		
<i>B. Coli communis</i> (Esch) -----	+		

With great probability

*Generally arabinose

Kelding E and Kelding T Method for the Demonstration of Small Quantities of Gold in Organic Substances *Acta Tub Scand*, 1925, 1, 200

The method described has been tested in 2000 cases and is claimed to be sensitive to 0.001 mg

Destruction of organic material Accomplished by evaporation and incineration of finely divided organs fecal matter blood, or urino

The ashes of the urine, organs, blood, and feces are dissolved by boiling three times with 10 cc of aqua regia and pouring all the solutions into one beaker The mixture is then diluted to 200 cc with water and evaporated to one fourth its volume

After cooling 50 cc of a 50 per cent solution of potassium carbonate and 25 cc. of an 8 per cent solution of sodium hydroxide are added and, shortly after, 50 cc of 5 per cent of sodium sulfite

Mix, heat to boiling, cool and filter

Acidulate the filtrate with concentrated HCl add 0.5 gm of potassium chlorate and boil for a few minutes

Precipitation of the gold

The solutions of organic ashes or urine thus obtained are diluted with water to 250 cc. in a 2 liter beaker and 200 cc of 8 per cent sodium hydroxide and 100 cc. of 1 per cent magnesium sulfate are added

The beaker is placed in a water bath at 65° C and while stirring rapidly, 50 cc of 30 per cent hydrogen peroxide is added. The temperature is maintained at 65° C for 2 hours and must not vary from 60° to 68° C. With a gold content of 0.5 mg or over, the addition of the hydrogen peroxide is at once followed by a red coloration of the magnesium hydroxide in the fluid. When small quantities—0.1 to 0.2 mg—are present, the color can be seen only when the precipitate has settled.

After two hours on the water bath the beaker is placed over the open flame and heated until all the hydrogen peroxide is boiled out (moderate boiling without violent bubbling).

Dilute with boiling water to 2 liters and allow to stand until the flocculent precipitate of magnesium hydroxide and gold has settled out. The color of the precipitate depends upon the gold content.

Siphon off the supernatant fluid and make up the volume of deposit to 100 cc.

Compare with standard gold solutions similarly treated.

Thomson, D., and Thomson, R. The Preparation of High Class Nutrient Media for the Cultivation of Germs Which Are Very Difficult to Grow. Ann. Pickett Thomson Research Lab., June, 1925, 1, 217.

The methods reported were elaborated during an investigation to find media suitable for the massive growth of gonococci for the preparation of large amounts of gonococcus vaccine during the World War.

It was found that the prolonged heating required in the usual method of preparing agar media produced an agar by product directly inhibitory to bacterial growth. The methods described are rapid and avoid overheating.

Methods—1 Preparation of agar solution. Agar fiber, sufficient to make a 5 per cent solution, is weighed and cut into short lengths. Add tap water and bring to a boiling point over a gas ring. The material must be constantly stirred to prevent burning. The resultant solution is invariably neutral.

2 Preparation of testicular bouillon with peptone and salts.

Ox testicles from the slaughter house are separated from their fibrous covering and minced as finely as possible in a meat grinder. The resultant fluid mass is weighed, an equal weight of tap water added, and the mixture slowly heated in an open vessel to the boiling point.

The heating should take from 20-30 minutes. When the boiling point is reached, quickly separate the coagulum by straining through a cloth. The resultant infusion is a pale, yellowish, milky fluid. It can be used at once or, after sterilization at 116° C in the autoclave, may be stored.

If stored and to be used for liquid media, the precipitate which forms should be removed by adjusting the reaction to P_H 4.5, heating to 60° C and filtering, or, preferably, by centrifuging in a Sharples centrifuge. If intended for a solid medium, removal of the precipitate is not necessary.

The clear fluid so obtained is brought to a P_H of 8.4 and the resultant precipitation of phosphates removed. After neutralization it may be used to prepare bouillon.

To a quantity of this testicular infusion add 4 per cent of peptone and 0.6 per cent of sodium dihydrogen phosphate. Ringer's salts may be used instead, if desired, the formula following:

Sodium chloride	90 gm
Calcium chloride	0.25 gm
Potassium chloride	0.42 gm
Sodium carbonate	0.3 gm
Distilled water	1000 cc

Ringer's solution does not appear to have any advantage over disodium hydrogen phosphate.

The peptone and salts are dissolved over the open flame by slow heating continued until the boiling point is reached.

Reaction adjusted to 1_R 77 in a comparator by using phenolsulphonephthalein as an indicator. After autoclaving for 30 minutes at 116° C the reaction will be decreased by P_R 02. This material is then added to the agar solution.

Rapid Process of Combined Sterilization, Clarification and Sedimentation. The apparatus devised by Mr. Downing is constructed as follows:

A metal can of one or two gallons capacity is secured consisting of a cylindrical body, a conical shoulder, and a cylindrical neck capable of holding a rubber stopper about 1¼ inches in diameter.

This stopper has two perforations in which are fitted two glass tubes, one reaching almost to the bottom of the vessel and serving as an air inlet, the other just long enough to reach above the layer of sediment which settles in the conical shoulder of the vessel when inverted. The length of this tube will naturally depend upon the shape and size of the can used. It provides outflow for the medium and is attached by a short length of rubber tubing to a hooded outlet. The flow is controlled by a Mohr clip.

To the air inlet tube a short glass tube plugged with cotton is attached by a short rubber tube and serves as an air filter.

The agar medium to be filtered is placed in the can which is plugged with cotton and placed in the autoclave. The rubber stopper with its attachments is wrapped in a towel and also placed in the autoclave.

A temperature of 116° C is maintained for 30 minutes when all are at once removed.

Sufficient 20 per cent sterile solution of glucose is now added to the medium to make a concentration of 0.5 per cent.

The stopper is inserted securely tied in and clips are attached to both rubber tubes.

The apparatus is then inverted and placed in a retort stand. The air filter, previously sterilized in the hot air oven is attached, the clip being removed. The entire apparatus is then placed in the Arnold adjusted to 60° C for 30 minutes to allow sedimentation to occur.

After the medium is thus clarified by sedimentation, the apparatus is removed, a new sterile air filter attached and the medium tubed or flaked.

The first few centimeters may be cloudy and are discarded. The remainder will be perfectly clear.

Enriching Fluids.—The most suitable are human body fluids such as whole blood, blood plasma, blood serum, hydrocele fluid, pleuritic fluid and ascitic fluid.

Blood is secured from patients requiring the Wassermann test and is kept from clotting by the addition of a small amount of 5 per cent sodium citrate. Either the whole citrated blood or the clear supernatant plasma may be used.

Blood clots from Wassermann specimens may also be used, the clots being broken up with glass beads and the resultant fluid boiled to sterilize it. Boiled blood is a very valuable enriching substance in the experience of the authors. If there is any doubt as to the sterility of the blood they heat it for one hour at 56° C.

Blood clots in the initial stages of decomposition may be used and, indeed, this medium containing the products of decomposition seems to possess certain advantages for bacteria requiring hemoglobin.

Hydrocele fluid is filtered through paper if a coagulum is present, the reaction adjusted to P_R 75 and a small quantity of Kieselguhr added. It is then filtered through a Berkefeld candle into sterile flasks for storage.

Addition of Enriching Substances.—A suitable amount—10 per cent—is placed in the sterile tubes or flasks and sterile agar added directly from the sedimenting vessel. For bacteria requiring hemoglobin the tubes or flasks are boiled for one minute and then cooled. This permits the use of blood which has become slightly septic. By the use of such media many organisms have been isolated from sputum apparently for the first time.

Fluid Media.—The advantages of the enriching substances described may be secured in fluid media by the following procedure. The enrichment substances are added to agar as a slant culture and the tube is then filled with testicular bouillon. The growth stimulating substances enter the bouillon by diffusion.

To obtain maximum growth the reaction must be kept neutral Phenolsulphon ephthalein is therefore added to the liquid medium and, as required, sufficient sterile sodium hydrate solution is added to bring out the pink color

It is suggested that the media described possess many advantages over those in routine use and should be on hand for everyday purposes

Cantero, A Bacteriology of the Thyroid Gland in Goiter Surg, Gynec, and Obst, January, 1926, p 61

Cantero reports a bacteriologic study of 50 goiters, mainly of colloid and adenomatous types Growth was obtained from all but three of the specimens

The tissues were examined immediately after removal After searing, a small portion was excised, washed in sterile N/S, emulsified in N/S and inoculated into various media including glucose brain broth and glucose brain agar Anaerobic cultures were also made

The brain broth was made from Difco dehydrated broth to which was added 0.2 per cent glucose, about 2 gm of calf's brain and several small pieces of marble

All media were adjusted to P_H 6.8 to 7.2, sterilized at 20 pounds for 20 minutes, and clarified by a continuous feed centrifuge

Cultures were incubated at $37^{\circ} C$ for 7 days and examined daily

Organisms belonging to the streptococcus group were isolated in 31 cases, pneumococci in 2, Welch's bacillus in 2, staphylococci in 7 cases, a diphtheroid, B pyocyaneus, and M tetragenus were each found once

The streptococci were of both hemolytic and viridans type

It is suggested that the localization of streptococci may be a factor in the pathogenesis of goiter

Burgess, J F On Some Aspects of the Cultural Study of the Ringworm Fungi Canadian Med Assn Jour, 1925, xv, 1003

By the microscopic examination of scales or hair stumps treated with 15 per cent potassium hydroxide the fungi may be classified as

1 Microsporon round spores 3 to 4 micra in diameter arranged in a mosaic around the affected hair stump

2 Trichophyton spores 3 to 4 to 5.7 micra in diameter, oval or oblong, and arranged in chains

3 Epidermophyton, found only in scales and seen as wavy, segmented strands

4 Achion irregularly segmented spore like elements seen in material obtained from the characteristic cup like lesions

Material for culture, if moist, is allowed to dry in sterile tubes for four to five days before culture

Hair stumps or scales are cut into small pieces with a sterile razor on a sterile slide and the pieces—5 to 6 to each tube—planted on 6 per cent glycerin agar Incubation is at room temperature and, while growth usually appears in 6 to 20 days, the rarer forms may require a period of 2 months

The morphology of cultures varies greatly from that of the fungus seen in lesions

It is necessary, for classification, to use a standard medium made with imported peptone

The formula for Sabourand's "media d'epreuve" is

Glucose C P	4 gm
French peptone (Chassaign)	1 gm
Agar	3 gm
Distilled water	100 c.c.

The imported peptone may be secured from the E P Dolby Co, Philadelphia, Penn
The "media de conservation" has the following formula

Peptone (Chnssaing)	3 gm
Agar	25 gm
Distilled water	100 cc

A large surface for growth is necessary and plants are made on the surface furnished by 60 cc of agar in 300 cc Erlenmeyer flasks

Pleomorphism after culture is common and transplants always yield pleomorphic varieties

Pleomorphism may be prevented by the use of the media de conservation after isolation

For microscopic study of cultures hanging drop preparations are prepared as follows

An ordinary rubber washer is sterilized by boiling and fixed to a sterile glass slide by sterile vaseline. A drop of 4 per cent glucose broth is placed in the center of another sterile slide inoculated with a small portion of the culture and the slide inverted over the washer the edges of which have been smeared with sterile vaseline. This forms an air tight chamber. From 15 to 20 such preparations should be made to compensate for failure of growth, evaporation etc

The guinea pig is suitable for inoculation on partially denuded areas lesions appearing in 7 to 12 days and disappearing spontaneously in 30 to 40 days

The article is illustrated

Kline B S and Young A M A Microscopic Slide Precipitation Test for Syphilis
Jour Am Med Assn, March 27 1926 LXVI: 928

The technic is as follows

Glassware and Apparatus—Ordinary microscopic slides are washed in soap and water, rinsed thoroughly in water, allowed to remain in 95 per cent alcohol for a short time, dried and then flamed. After this four paraffin rings (each with an inside diameter of 11 to 12 mm) are made on one surface according to the method of Green by transferring a small amount of hot paraffin on a stiff wire (gage 19) wound with thread (or hat wire) bent to the form of a circle

The pipettes needed for delivering the serums are the ordinary 1 cc pipettes graduated in 0.01 cc. The pipettes for the antigen are the same as those for the serums with the ends drawn out so that each drop of antigen equals 0.015 cc. The diameter of the tip over all is about 1.25 mm

Vials for preparing the antigen dilution are similar to those used and recommended by Kahn

A humidifier is necessary for the test. The one employed in this laboratory consists of a wooden lid, 16½ by 4 by 1½ inches inside diameter, with a moistened blotter fastened in place with thumb tacks

Antigen—The antigen and antigen dilution are prepared as for the Kahn test. The antigen extraction likewise is done as for the Kahn test. The antigen dilution should be made up just before pipetting the serums. Some antigen dilutions have been found to work only within fifteen minutes of their preparation. An average antigen dilution may still be used forty five minutes after its preparation. The action of the antigen dilution has been found unsatisfactory when the room temperature and that of the microscopic slides is low again, false clumping occurs in serum antigen dilution mixtures allowed to dry. Accordingly it is important to do the test in a warm, humid room (about 80° F with visible moisture on the windows), which is readily prepared by heating a pan of water after closing the windows and doors. The table top on which the tests are performed should be kept warm. A piece of harness felt three fourths inch thick is satisfactory for this purpose

Serums—These are obtained as for the Wassermann test, care being exercised that they contain no red blood cells or foreign material. Before use, they are heated to 56° C for one half hour

appendicitis, peritonitis, strangulated hernia, intestinal obstruction, infection of the uterine cavity, ruptured ectopic, eclampsia, tetanus, and acute acidosis from any cause

Those diseases producing a leucocytosis in which all the various types of leucocytes are more or less increased include the following acute salpingitis and infection of the ovary, pyelitis, cystitis, infection of the prostate gland and other organs or parts of the male genitourinary tract, hepatic colic, and practically all acute infections of tissues outside of the abdominal cavity and not mentioned in the previous classification

Ecker, E E, and Megraill, E Production of Toxic Substances in Young Cultures of Single Cell Strains of *B Paratyphosus B* Jour Infect Dis, December, 1925, xxxvii, No 6, 546

Berkefeld N filtrates of 2 per cent Witte peptone veal infusion broth cultures of 5 single cell strains from each of two cultures of *B paratyphosus B* injected intravenously into rabbits proved to be as toxic as the filtrates of parent cultures Boiling for three minutes did not lessen the toxicity No significant cultural differences were noted between the single cell and parent strains

Brown, H C, Duncan, J T, and Henry, T A The Differentiation of Food-Poisoning Bacteria The Lancet, London, Jan 16, 1926, p 117

The salts below are suggested as an additional means of differentiating organisms of the *Salmonella* group of bacteria

Peptone water containing one per cent of the sodium salts of citric, d tartaric, l tartaric, m tartaric, fumaric and mucic acids, is used as the culture medium

After incubation of the cultures for 18 to 96 hours 0.6 cc of a saturated solution of lead acetate is added for each 5 cc of the tartrato or citrate medium

All of the acids yield insoluble lead salts and decomposition of the acid salts is evidenced by a decreased precipitation

Incubation of the fumaric acid medium should be continued for 96 hours as decomposition is slow

With mucic acid a little acetic acid should be added to dissolve lead carbonate which may form and so distinguish it from lead mucate

The reactions obtained are indicated below

	CITRATE	D TARTRATE	L TARTRATE	M TARTRATE	FUMARICITE	MUCATE
B para A	-	-	-	-	-	-
B para B	+	-	+	-	-	+
B para C	+	+	-	+	-	-
B Supester	+	+	-	+	+	-
Salmonella type G	+	+	-	+	+	-
Type Reading	+	+	+	+	+	+
Type Mitten	+	+	+	+	+	+
Type Newport	+	+	+	+	+	+
Type Binns	+	+	+	+	+	+
Type Derby	+	+	+	+	-	+
B Gaertner	+	±	±	±	+	+

Rockwell, G E, and Highberger, J H Carbon Dioxide as a Factor in the Growth of the Tubercle Bacillus and of Other Acid Fast Organisms Jour Infect Dis, January, 1926, xxxviii, 92

It is shown that the inhibition of growth of a saprophytic tubercle bacillus, two strains of virulent tubercle bacilli, and two other acid fast organisms, when incubated over alkalis in closed spaces, cannot be explained as due to dehydration of the medium, since growth occurs over more efficient dehydrating agents which are not carbon dioxide absorbents, such as sulphuric acid, calcium chloride, and glycerine The only explanation of this phenomenon is that carbon dioxide in some way is a vital factor in growth

Rhea, L J Stand for Staining Blood Smears Internat Assn Med Mus, 1924, p 91

The author describes a stand for staining blood smears which is very simply prepared with the equipment in any laboratory and has the advantage of preventing evaporation of the alcohol during the staining with Wright's stain

A wide mouthed bottle is fitted with a cork in which a hole is cut, just large enough to fit snugly into the glass knob of the cover of a glass jar. In the base of this inverted cover two narrow platforms of glass are cemented at a convenient distance apart with reference to the ordinary glass slide and slightly narrower than such a slide. The slide with the smeared preparation of blood to be stained is placed on these platforms, flooded with the Wright's stain in the usual manner and the whole covered with a pane of glass. It is advisable to have a little methyl alcohol lying free in the base of the inverted cover so that during the staining process an atmosphere saturated with the alcohol is present and no evaporation occurs from the staining fluid

White O P A New Method of Decalcification Jour Path and Bact London 1923
xxvi, 425 Abstr Bull VI Internat Assn Med Mus May 4, 1925

A saturated watery solution of citric acid is diluted 1:10 with water. A small quantity of methyl red and naphtholphthalein are added and then strong ammonia until the fluid is a clear yellow color. Too much ammonia turns the fluid green. The fluid contains about 6 per cent citric acid and should have a P_H of 6 to 8. Chloroform is added to prevent the growth of moulds. After decalcification wash well in water before placing in alcohol.

The advantages are the use of a neutral solution for decalcification. The staining is unaffected and it seems to exert no harmful effect on the tissue.

Smith J L and Rettie T An Aldehyde Mordant for Fats and Lipoids Jour Path and Bact, 1924, xxvii, 115 Abstr Bull VI Internat Assn Med Mus May 4 1925

Preparation of the aldehyde solution

In a flat bottomed 400 cc flask put 20 cc of paraldehyde. To this add 25 cc of dilute HCl (equal parts water and pure HCl). Heat in an oven at 37° C with frequent shakings until the paraldehyde is dissolved. This takes fifteen hours or longer and it is not safe to leave in the incubator overnight. The resulting pale brown solution is diluted with water neutralized with NaOH and made up to 1000 cc (25 per cent paraldehyde). Adjust reaction to P_H 6 with acetic acid. Stored in dark bottles it keeps for months and reaction should be readjusted if changed to P_H 6 with caustic soda. The tissues to be stained are fixed in 10 per cent formalin for twenty four to forty eight hours and not longer. Frozen sections are cut and mordanted in this solution at 37° C for twenty four to forty eight hours. Wash in water and stain six to eighteen hours in 1 per cent hematoxylin in 0.5 per cent acetic acid. Differentiate in solution containing 1 per cent horax and 0.5 per cent potassium ferrieyanide. The fat and lipid globules alone remain dark blue.

Hirschfeld H Experience with Oxidase and Peroxidase Reactions Med Klin Berlin, 1924, xx, 249 Abstr Bull VI Internat Assn Med Mus May 4 1925

The most convenient method of applying the oxidase reaction is that of W H Schultze

Fixation of blood preparation in absolute alcohol and then treatment for about five minutes in a filtered mixture of equal parts of a 1 per cent aqueous solution of basic dimethylparaphenylenediamine (Merck or Schuchardt) and a 1 per cent strongly alkaline or alcoholic solution of alpha naphthol.

The older the solution the shorter will be the time necessary for fixation. Finally, however, the dimethylparaphenylenediamine especially loses its action. If one wishes to preserve the preparations thus treated and counterstained with safranin, fuchsin or pyronin, they must be embedded in water glass but even then their durability is limited.

For the peroxidase reaction Graham's modification has been found useful

Fixation for about half a minute in a mixture of 1 part formaldehyde and 9 parts 95 per cent alcohol. Carefully wash off and then place for five minutes in a solution of a few grains of benzidine in 40 per cent alcohol to which 10 cc of 0.02 H₂O₂ have been added

Staining follows in Loeffler's methylene blue or better, a highly concentrated Giemsa solution (ten to fifteen minutes' staining in a solution of 0.6 Giemsa in 10 cc Aqua destil)

This produces many beautiful preparations in which all normal and pathological leucocyte forms are easily differentiated

Kinney, E W, and Campbell, H Jahnel's Method for Staining Spirochetes in Nerve Tissue Bull XI Internat Assn Med Mus, May 4, 1925, p 121

The method following has given excellent results in tissues preserved for some time in formalin, but is not so satisfactory for freshly fixed tissue. The best results were obtained in tissues preserved for years (ten)

1 Wash pieces of formaldehyde or alcohol fixed tissue from 2 to 4 mm thick in distilled water for one to three days

2 Pure pyridine one to three days

3 Wash for two to three days in many changes of water until the pyridine odor almost disappears, this is important

4 Allow the pieces to remain a "few days" (einige tage) in a 5 to 10 per cent formaldehyde solution, USP

5 Place in water again (The time in water is not stated here, probably the washing should be thorough)

6 Place in a 1 per cent solution of uranium nitrate (Merck) in distilled water for one half to one hour in the incubator at 37° C. Lead free glass wool may be used under the tissue to aid penetration but is not absolutely necessary. The uranium nitrate inhibits the staining of other elements of the nervous tissue

7 Wash in distilled water for one day

8 Ninety six per cent alcohol three to eight days

9 Distilled water until the block sinks

10 Freshly prepared 15 per cent silver nitrate solution in an amber flask from five to eight days in the oven at 37° C

11 Decant the silver nitrate solution, wash the tissue slightly in water and transfer to the following solution for one to two days

4 per cent aqueous solution of pyrogallol	95 cc
Formaldehyde solution, USP	5 cc

"We have found it unnecessary to leave the tissue in any solution longer than the minimum time given. In Step 4 we leave blocks twenty four hours in the formaldehyde solution. In Step 5 we wash twenty four hours in frequently changed distilled water."

Of the brains examined which had an anatomic and histologic diagnosis of general paresis 58.3 per cent showed spirochetes by this method

Boissevain, C H A Method for Obtaining Single Colonies of Tubercle Bacilli. Am Rev Tub, January, 1926, xiii, 90

"For the study of variation of virulence, or any other biological property of bacteria, it is important to use cultures derived from a single organism. Ordinary cultures, made by planting millions of bacilli and securing a massive confluent growth, are likely to contain a mixture of strains which may differ widely from one another. With organisms which grow vigorously on artificial media it is easy, by simple planting, to get colonies, which we are reasonably sure have grown from one individual, or we may use the Barber Technique (1) and be quite certain, but with the tubercle bacillus such methods usually give no growth. In the hope of finding a satisfactory method the following procedures have been tried, with encouraging results

"A" A rabbit is bled from the carotid artery and the blood received into paraffined centrifuge tubes packed in ice, and centrifuged at once. A rather light suspension of tubercle bacilli in 15 per cent sodium chloride solution is prepared, filtered through sterile filter paper and centrifugated for a few minutes. One drop of the supernatant suspension is mixed with 1 cc of the rabbit plasma in a sterile tube stoppered with paraffined cotton slanted to form a thin layer and left to coagulate in the incubator. Coagulation occurs in about ten minutes and after that the tubes should be left lying on their sides. In an upright position all the serum is pressed out of the coagulum and collects at the bottom of the tube. In about two weeks many small discrete colonies can be seen in the plasma but not on the surface. The culture can be transplanted or used at once for inoculation. This confirms an observation of A. E. Wright on the multiplication of tubercle bacilli in capillary tubes filled with plasma.

"B" Rabbit serum with the addition of an equal volume of 3 per cent agar in distilled water at 50° C but without peptone salt or glycerine is substituted for the plasma. With this method the results have varied. Serum from some rabbits has yielded a few colonies, serum from others none. In no instance has the growth been as abundant as with plasma.

"C" Freshly ground rabbit liver is added to the rabbit serum of method B in the proportion of about 1 to 20.

"In this medium the tubercle bacilli grow as well as in plasma. The liver of a tuberculous guinea pig may be used and primary growth be secured in the form of single colonies. There would, however, be more doubt of the colonies having developed from a single bacillus than when a light filtered and centrifugated bacillary suspension is used."

"D" In 'hormone agar plus rabbit serum in equal parts colonies develop abundantly. If the serum is omitted a few colonies sometimes appear. Experiments are now in progress to ascertain what substance present in tissue and in plasma is so favorable to the growth of the tubercle bacillus."

Hackenthal H. A Modified Schuffner's Blood Stain. *Deutsch Arch f klin Med* Leipzig 1924 cxviii 276

The author gives a staining method designed to avoid the drawbacks of the usual smear preparation. Destroyed or artificially distorted cells are unusually few and cells and nuclei maintain their living form. All the cells which are differentiable in the smear preparation are also easily distinguishable.

Technic

Schuffner's Solution

NaCl	40
Borax	0.1
Concentrated carboic acid	30
Formalin	10
Aqua destil	10000

To every 2 cc of Schuffner's solution 1 to 2 drops of saturated aqueous methylene blue solution (methylene blue medicinal Hoechst) and 1 drop of saturated aqueous dahlia solution (G. A. Hesterberg, Berlin Lindenstrasse 39). One-tenth cubic centimeters of this solution, which must be freshly made up, is placed in a small cylindrical glass vessel made from a 4 or 5 cm long piece of glass tubing of 0.5 to 0.7 cm bore. To this add 20 cmm of blood using a capillary pipette from a Sahli's or Gower's hemoglobinometer which must first be washed out with Schuffner's solution or the greater number of the leucocytes will stick to its walls. A glass capillary tube first washed through with Schuffner's solution is used to mix the blood and stain thoroughly. A small drop of the mixture is placed on a clear slide and covered by a cover glass. The drop must be of such a size that it will reach to the edge of the cover glass and yet the cover glass must not float on it. The edge of the cover glass is then 'ringed' with vaseline to hinder evaporation and the resulting injury to the blood cells. The blood cells will now lie almost in an optical plane

without streaming movements In warm weather staining of the leucocytes proceeds more quickly than in cold weather

Erythrocytes remain unstained except those which in smear preparation appear darkly colored as in metachromasia, these become light blue to light violet Nuclear fragments of erythroblasts, stippling inclusive of Schuffner's stippling in malaria are well stained Poikilocytosis and anisocytosis are recognizable without exception

Hall, M W, and Lacy, G R The Mechanism of the Russell Double Sugar Tube Jour Infect Dis, January, 1926, *xxxviii*, 14

While the acids produced in the fermentation of dextrose in the Russell double sugar medium are mainly, if not entirely, volatile, their diffusion out of the medium is not the cause of the alkaline reaction shown on the slant when typhoid and related organisms are grown This reaction is due to excess of alkaline substance produced from nitrogenous elements in the presence of oxygen

In the absence of oxygen a "mother substance" is formed which rapidly becomes alkaline when exposed to oxygen

The ultimate reversion in the butts of Russell tubes is the result of the diffusion into the butts of oxygen rather than of the alkali formed in the slant portion of the tube

The paratyphoid and colon groups appear capable of producing an alkaline substance under anerobic conditions, thus evidencing a distinct difference in metabolism which may prove worthy of further study The chemical nature of the substance which is so easily oxidized into an alkaline substance is unknown

Stevens, F A, and Dochez, A R Occurrence of Throat Infections with *Streptococcus Scarletinae* without a Rash Jour Am Med Assn, April 10, 1926, *lxxxvi*, 1110

A study of an epidemic of streptococci (hemolytic) sore throat during 1924-25 in a hospital demonstrated that

- 1 Scarletinal infection of the throat may occur without a rash
- 2 This type of infection may occur in individuals showing negative skin reactions to scarlatinal toxin
- 3 The Dick test is not a reliable index of immunity to such throat infections with *streptococcus scarlatinae*
- 4 In the series of cases observed agglutination reactions with scarlatinal serum and toxin production are closely parallel
- 5 There is no antigenic relationship between strains of hemolytic streptococci from acute streptococcus pharyngitis

Hirsch, E F Separation of a Soluble Specific Substance from Hemolytic *Streptococci* Jour Infect Dis, December, 1925, *xxvii*, No 6, 523

The method used was as follows

Broth was prepared with fresh placenta tissue, 1 pound of the minced tissue per liter of water To the filtered portion, 1 per cent peptone and 0.8 per cent sodium chloride were added After heating to boiling, the reaction was adjusted to P_H 7.6, the mixture filtered and sterilized To each 100 cc by volume, approximately 20 cc of sterile aseptic fluid was added Containers of this medium were inoculated with a strain of *Streptococcus hemolyticus*, incubated at 37° C for 48 hours, and the growth of bacteria recovered by centrifugation The sediment so obtained was washed quickly in 0.9 per cent salt solution To the bacteria N/10 NaOH was added, (enough to make a turbid suspension), and the mixture transferred to a 250 cc separatory funnel Small quantities of ether added to this were thoroughly shaken into the mixture, and presently formed with the bacterial suspension a fairly stable emulsion The emulsion so started was built up by adding small quantities of ether and shaking When the amount of ether added exceeded that capable of remaining emulsified, the excess was removed, and the separatory funnel with its content stood upright for about 1 hour At the end of this time two layers

formed in the funnel—a lower clear layer and an upper gummy layer. The clear layer was removed and the gummy layer above containing the emulsified ether, was broken up by rotating the separatory funnel. The ether, separating by this manipulation was poured off or evaporated with a current of air. This left a turbid liquid which was filtered, the filtrate centrifugalized and the clear or slightly opalescent liquid electrodyalized against distilled water. When the reaction of the liquid came to the proper P_H by electromeasurement and adjustment with dilute acid or alkali there was a turbidity and on standing a copious flocculation of white particles. A reaction slightly acid or alkaline stabilized the particles and there was no flocculation. However the acid solution was more opalescent than the alkaline. The white flocculent precipitate was recovered by centrifugation washed repeatedly in distilled water used as such or dried in a vacuum desiccator. Washing with distilled water usually results in a loss of some material because the P_H of the distilled water used in washing does not coincide with the P_H (isoelectric) of flocculation. Finally the washed material may be reduced to a brown white powder.

The material so obtained is soluble readily in alkaline solutions, and in this respect corresponds with extracts obtained from other bacteria. It is also soluble to a less extent in dilute acids (N/100). Increasing the acidity renders the solution more opalescent and heating seems to change the solubility in acid solutions. The moist precipitate is slightly soluble in 0.9 per cent sodium chloride solution it is insoluble in 90 per cent alcohol acetone and ether. A solution of the precipitate in water containing a few drops of N/100 sodium hydroxide reacts with Millon's protein test but not with the biuret test. There is no reduction of Fehling's solution no precipitation with 95 per cent alcohol or acetone. On heating alone and with dilute acetic acid there is a slight haze. One preparation not regarded as entirely pure contained about 10 per cent nitrogen. A solution gave precipitation reactions with an antistreptococcus rabbit serum in dilutions as high as 8 parts in 100,000. This antistreptococcus immune serum has only a moderate titer. Solutions of many preparations made in that way gave precipitation reactions with antistreptococcus serums and with the serum of a rabbit receiving repeated injections with solutions of other similar preparations. There is no hemolysis when isotonic solutions of these preparations are mixed with red blood corpuscles.

DeAza E. A. S. *Gonococcus Vaccines with Urotropin*. Rev. Med. Barcelona October 1925 iv, 374.

The author reports his experience with vaccines preserved with urotropin which apparently delays the aging of vaccines and preserves them.

It was found that vaccines with urotropin gave in small doses a much stronger reaction than plain vaccines. The small dose of urotropin incorporated in the vaccine acts solely by preserving the initial strength of the vaccine in other words such vaccine keeps fresh for a much longer period.

The proportion of urotropin in the vaccine is not stated.

Garrod L. P. *On Sulphemoglobinemia*. Brit. Quart. Jour. Med. October 1925 vii, 86.

In two cases of sulphemoglobinemia the feces gave a growth of the nitrosobacillus and treatment with a vaccine of this organism produced prompt results.

This method used for isolation of the organism was as follows:

A weighed amount of feces is completely emulsified in sterile distilled water and a series of dilutions made from each of which 0.1 c.c. was plated on litmus lactose agar plates. The plates should be spread for at least 30 seconds to permit complete absorption of the fluid by the medium.

Three such series of plates were made there being six in each series representing amounts of feces from 1/50,000 to 1/10,000,000 grm. The plates were then incubated at 37° C, 30° C, and 20° C. In those incubated at 37° C only colon bacilli and streptococci were obtained, in the others the coliform colonies are smaller and the nitrosobacillus colonies appeared on the fourth day.

The organism was an oval, gram negative, nonmotile bacillus, growing slowly on all media, fermenting no sugars, reducing neutral red, liquefying gelatin, and forming nitrites
The author urges a search for this organism in similar cases

Falk, I S, and Yong, S Y The Influence of Certain Electrolytes and Nonelectrolytes on the Bile Solubility of Pneumococci Jour Infect Dis, January, 1926, xxxviii, 1

Washed suspensions of pneumococci in distilled water are usually bile soluble

Chlorides with monovalent cations (Na, K, NH_4 , Li) in relatively low concentrations inhibit bile solution of washed pneumococci

In higher concentrations these chlorides do not inhibit and may accelerate solution

Chlorides with divalent cations (Ca, Ba) behave differently and inhibit bile solution of pneumococci more effectively in high than in low concentrations

Of the anion series tested NaOH and Na_2PO_4 are cytolytic for pneumococci, NaHPO_4 , NaH_2PO_4 , Na_2SO_4 , and NaNO_3 are not cytolytic

Cytolysis by NaOH and Na_2PO_4 appears to be a function of pH concentration

Peptone, gelatine, and ovalbumin appear to inhibit cytotoxicity by bile in the same manner as CaCl₂ and BaCl₂ The inhibitory action increases with concentration

Kerr, D, and Mason, V H The Hemochromogen Crystal Test for Blood Brit Med Jour, Jan 23, 1926, p 134

The advantages of Takayama's method for the detection of blood by the formation of hemochromogen crystals are extolled

The test solution consists of 10 per cent sodium hydroxide solution, 3 cc, pyridin, 3 cc, saturated solution of grape sugar, 3 cc and distilled water, 7 cc

The sodium hydroxide acts as a blood solvent and the grape sugar as a reducing agent The solution keeps for about two months

On the addition of two or three drops of this solution to a small piece of the suspected material on a slide in the cold and covering with a cover glass, salmon pink crystals appear within two or three minutes which can be clearly seen under the low power (200 to 300 magnifications)

At the same time the color changes through green brown, dark red, to pink thus indicating the formation of hemochromogen and confirming the test

The crystals usually appear within one to six minutes but a negative test should be observed for 30 minutes

If the slide be heated just to bubbling the crystals appear almost at once

There is no danger of overheating

The crystals are single, shallow, rhomboids of salmon pink color When on their side they appear like dark, single lines

They are often very large

The crystals may be obtained from material which has been heated, washed or contaminated with rust

Milroy, G A Method for Estimation of Glucose in the Blood Jour Biochem, 1925, xiv, 746

1 Precipitate the proteins from 1 cc of blood by the Fohn Wu method The blood is, hence, diluted 1:10

2 After standing 30 minutes, filter and place 5 cc of filtrate in a 15 cc graduated tube

3 Into five graduated 15 cc tubes of 12 mm bore introduce the following amounts of 0.02 per cent glucose solution 15, 18, 21, 25 and 3 cc

4 Add to each tube 1 cc of 0.4 per cent aqueous 1:5 nitroanthraquinonesulphonic acid and 2 cc of potassium carbonate solution (50 mg per 100 cc)

5 Dilute all tubes to 10 cc mix, immerse in boiling water for 8 minute, cool and make the volume 125 cc

6 Compare the red tint of the blood filtrate with the glucose solutions

The minimal error in a sample of blood containing 0.1 per cent glucose is 4 per cent

Belding D L. Notes on the Etiology and Epidemiology of Impetigo Contagiosa Neonatorum. Am Jour Obst and Gynec January 1926, 21, 1

The study corroborates the etiologic relation of a strain of *Staphylococcus aureus* to impetigo contagiosa neonatorum. The strain failed to produce skin lesions in guinea pigs or rabbits but caused a nonvesicular inflammatory reaction in the skin of an adult and a typical exfoliating lesion in the skin of the infant from whom it was isolated. The cultural reactions, except for a questionable minor difference in the rate of carbohydrate fermentation, are the same as those of the ordinary *staphylococcus*.

The variation in epidemics is probably due to variations in the virulence of the infecting strain, variations in the clinical symptomatology of infants and adults are probably due to the resistance of the host.

The primary prophylactic measure in a hospital epidemic is the individual handling of the well infants, as early cases are capable of transmitting the infection before a diagnosis is made.

Joeke Th. Cultivation of the Spirillum of Rat-Bite Fever. The Lancet London Dec 12, 1925

Successful results were obtained with a medium consisting of Loeffler's blood serum on top of which was poured 10 cc of Vervoort's medium (peptone 1 gram, normal phosphoric acid 3 cc, distilled water 900 cc).

The most favorable reaction is P_{H} 7.2 to 7.6. The reaction of Vervoort's medium is originally P_{H} 6.6 but, after remaining for 15 minutes in contact with the serum slope at 37 C alters to P_{H} 7.0 to 7.2.

Sanders, G H. Isolation of Monilia from the Skin Scales Mouth and Sputum in Psoriasis. The Lancet, London, Dec 12 1925

An organism belonging to the monilia was isolated in conjunction with streptococci from the areas noted above.

Tunnichiff, B and Hayne A L. Further Studies on a Diplococcus from Measles. Prevention of Measles by Immune Goat Serum. Jour Infect Dis January 1926 xxxviii, 1, 48

Report of further studies on a filter passing gram positive green producing diplococcus isolated from the blood in the early eruptive stage of measles by Tunnichiff in 1917.

The present paper reports the successful prevention of measles in rabbits by the injection of immune goat serum.

Convalescent goat serum also protected human beings against measles as effectively as human convalescent serum when injected on the first and second days after exposure.

Holman W L. An Error in Acid Fast and Gram Staining Due to Petrolatum. Arch Path. and Lab Med, March, 1926, 1 No 3, p 390

Smears made from lesions in which petrolatum has been used must be treated with xylol or some other solvent before staining. If this precaution is omitted false gram positive or acid fast reactions will be obtained.

The possibility of this error must be recognized when petrolatum has been used on catheters or when smears are made from anaerobic tubes in which petrolatum is used on the surface of the medium.

Ramos, Passos J R On Lowenstein's Procedure for Direct Isolation of the Tubercle Bacillus from Sputum Compt rend Soc Biol, Paris, Dec 18, 1925, xciii, 1552

Technic—The sputum is placed in a centrifuge tube and five times its volume of a 15 per cent solution of sulphuric acid is added. The tube is allowed to stand for 20 minutes, shaking occasionally until a homogenous mixture is obtained. At the end of this time the sputum is centrifuged at high speed for 15 minutes. The liquid is removed and the sediment is washed with sterile physiologic salt solution. The centrifugation and washing are repeated two more times. The liquid in the tube is then cultured on glycerinated potato, five tubes of the medium being used for each specimen. The tubes are well stoppered and paraffined and are then placed in the incubator at 37° C.

The method proved successful in the hands of the author in 104 microscopically positive and in two out of 33 microscopically negative specimens.

Tunnichiff, R Further Studies on a Diplococcus in Measles. A Measles Skin Reaction. Jour Infect Dis, September, 1925, xxxvii, No 3, p 193

Anerobic dextrose broth cultures of a diplococcus found by the author in measles, killed by 0.5 per cent phenol, appear to produce a skin reaction in persons who have not had measles, but not in measles patients after the appearance of the eruption, or in 96 per cent of persons who give a history of measles.

The measles antigen is neutralized in persons who have not had measles by convalescent human measles serum, but not by the serum of a person with a negative history of measles.

Rabbits immunized against measles fail to react.

Greenough, R B Varying Degrees of Malignancy in Cancer of the Breast. Jour Cancer Research, December, 1925, iv, No 4, 453

A report of a study of cases of cancer of the breast in the Massachusetts General Hospital during 1918, 1919 and 1920 in accordance with the principle of McCarty and Broders that the degree of malignancy may be estimated by loss of differentiation and increase of productive characteristics, dividing the tumors into three groups of high, medium, and low malignancy.

The results are thus summarized:

1 The degree of malignancy of a given case of cancer of the breast can be determined with reasonable accuracy by study of the histology.

2 A classification of high, medium, and low degrees of malignancy can thus be made which is of prognostic value and of aid in the evaluation of therapeutic measures.

3 In estimating the degree of malignancy the following factors are of importance:

(a) Degree of differentiation, as shown by arrangement of cells around an open gland, (adenocarcinoma).

(b) Degree of secretory activity of cell protoplasm as shown by vacuoles and droplets of mucoid material.

(c) Uniformity of size of cells and nuclei as opposed to variation in size.

(d) Absence or presence of hyperchromatic changes in nucleus and few or many mitotic figures, and whether irregular or not.

(e) High malignancy is shown by cells and nuclei of irregular shape and size without secretory function, and arranged in solid columns, large or small, together with numerous and irregular mitoses and hyperchromatism. The extreme degree of these changes is pleomorphism.

(f) A tumor of adenomatous arrangement with uniform sized cells and nuclei, few mitoses, and absence of hyperchromatism, indicates low malignancy.

4 A high degree of round cell infiltration appears to indicate a considerable degree of cell degeneration and is not to be relied upon as an indication of the resistance of the individual to the cancer growth.

5 Hyalinization of the stroma does not indicate active resistance to the tumor growth but is rather a factor of the age or previous condition of the mammary tissue in which the tumor lies

The paper is illustrated with sixteen microphotographs showing the appearances described

Cruikshank R. B. *Bifidus* Its Character and Isolation from the Intestine of Infants
Jour Hygiene December 1905 *xxix* 211

The following method was used for the isolation of *B. bifidus* from the feces

A deep tube containing 20 c.c. of 1 per cent lactose or glucose broth neutral to litmus, and containing a small piece of fresh sterile rabbit kidney and sealed with vaseline is inoculated by means of a capillary pipette with 0.5 c.c. of a fairly opaque fecal emulsion. The vaseline is melted to seal the track of the pipette and the tube incubated 6 to 8 days at 37° C.

During the first few days the gas formed is conveniently expelled by melting the vaseline seal.

Plates of 1 per cent glucose agar or Loeffler's serum are streaked and incubated aerobically and anaerobically.

After 48 hours at 37° C. small glistening grayish colonies of pin head size are seen, generally on the anaerobic plate occasionally aerobically.

The only other organism likely to be found is the enterococcus which forms larger whitish colonies readily distinguishable from the diphtheroid *B. bifidus* colonies.

Subcultures are made aerobically in glucose agar slants, glucose broth and milk. Rich inoculation is advisable.

Harris J. S. A Simple Test of Diagnostic Value in General Paresis. *Brit Med Jour*
 Jan 23, 1920 p. 136

Harris reports his results with the acetic anhydride sulphuric acid test described by Grossman in 1925 (*Jour Mental Sc.* *xxi* 439).

The method follows:

To 1 c.c. of spinal fluid in a small test tube add 0.3 c.c. of acetic anhydride. Shake well and add carefully, drop by drop 0.8 c.c. of concentrated sulphuric acid. The tube is then held against a white background. A lilac tint indicates a positive reaction; a brown yellow or red yellow constitutes a negative reaction. The lilac color of a positive reaction may appear and disappear within a minute or so; hence the reading should be made at once.

The test is presumed to be due to the presence of cholesterol in the fluid.

In 180 cases of various mental diseases Harris found this reaction positive in 97 per cent of paresies and negative in all the control conditions.

Baer J. L. and Reis E. A. The Sedimentation Test in Obstetrics and Gynecology
Surg Gynec and Obst May 1925 p. 691

Using the technique described by Lanzetta the authors report a study of the sedimentation test in 100 cases of pregnancy and in various gynecologic conditions.

They conclude:

1. The sedimentation test is of no value in the diagnosis of pregnancy.
2. With pelvic pathology a sedimentation time over 2 hours conclusively rules out pelvic infection.

3. The rate of sedimentation is directly proportional to the virulence of the infection.

4. The test is of value in determining the safe time for operation and seems a more delicate prognostic index good or bad than either the leucocyte or temperature curve.

(A further communication by the same authors—The Sedimentation Test in Gynecology
Am Jour Obst and Gynec September 1925 *x* 3—was abstracted in the January, 1926 issue of this Journal.)

Separate sections are devoted to special aspects of hygiene and sanitation (33 pages), demography (25 pages), and a final section on public health administration (11 pages)

The volume amply fulfils its purpose and should be in the hands of every practitioner

*The International Association of Medical Museums**

THIS eleventh bulletin of The International Association of Medical Museums within a limited space comprises a storehouse of information of interest alike to pathologists, curators of museums, and technicians

In addition to a report of the various activities of the association, there are twenty original articles, covering a variety of subjects from the histologic findings of a tumor preserved in alcohol for 118 years to new or improved technical procedures

There is also a very extensive and useful department of technical abstracts. The publication very ably reports the activities in its special field

Memoranda of Toxicology†

BASED upon Tanner's *Memoranda of Poisons*, this little handbook serves a distinct purpose

After a general consideration of the mode of action and classification of poisons, including some very clearly written chapters on the diagnosis of poisoning, the duties of the practitioner in cases of poisoning, and the treatment and detection of poisons in general, various irritants and poisons are then considered in detail

The diagnosis and treatment of poisoning is a subject of some difficulty and while there are many books available on this subject not all are sufficiently compact or concise to furnish readily available information

The present volume in a small space presents a large amount of eminently practical information

The newer sources of poisoning, such as tetraethyl lead, asphyxiating gases, barium salts (as used in x-ray work) are included and all the newer data concerned with diagnosis and treatment has received consideration

Each poison is briefly but satisfactorily discussed under the following headings: Nature of Action, Symptoms, Postmortem Appearances, Treatment, and Tests. While it is beyond the province of the book to discuss in detail the chemical detection and determination of poisons, under "tests" are given ready and simple methods for their general recognition

Addenda, contributed by Dr. Leffman, include discussions on Bites and

*Bulletin No. XI of The International Association of Medical Museums and Journal of Technical Methods. Pp. 151. Illustrated Paper. Price \$3.00. Paul B. Hoeber, Inc., New York.

†*Memoranda of Toxicology*. By Max Trummer, A.M., formerly Lecturer on Toxicology, Jefferson Medical College, and Henry Leffman, M.D., Emeritus Pathologic Chemist, Jefferson Medical College. Flexible pocket size. Pp. 230. Price \$1.50. P. Blakiston's Son and Co., Philadelphia.

Stings, Drug Addiction, The Poison Rum Problem, First Aid, What Is a Poison, and Tobacco as a Medium for the Administration of Patent Drugs

For the medical student and industrial or general practitioner this little book will be most useful

*Aviation Medicine**

THE specialty of aviation medicine represents almost entirely a development of the last twenty years. The present volume is the first textbook written on this subject in America, and the first one written anywhere since the late war. Great progress is constantly being made along the lines of aviation, and its probable future importance in civil as well as in military life renders this volume of peculiar interest as a kind of practical pioneer in a subject which has enthralled the human mind from the time of its earliest conception of birds or angels or dragons or other similar creatures which could soar in the air, often to the disadvantage of man, and always with the excitement of his envy.

The author has attempted to consider all phases of the subject, to correlate the results of various workers and to present a handbook for the use of those interested in the subject. It is intended that the book shall be sufficiently technical to be of use to the Flight Surgeon, but not too technical to interest the physician who has not worked in the subject.

The book is made up of three sections, the first of which deals with the selection of the flyer, the second with the physiology of aviation including the classification of the flyer, and the third with the care and maintenance of the flyer.

The book contains seventeen chapters, a supplement and a splendid bibliography of some twenty six pages. A summary conception of the wide range of the subject matter involved may be obtained from a brief consideration of the chapter headings which are as follows. Section one contains chapters on general physical qualifications, the eye in aviation, the nose, throat, and ear in aviation including a consideration of equilibrium neuropsychic factors and reaction time. Section two contains chapters on the effects of altitude on the respiratory and circulatory systems, the effects of altitude on the heart, psychological effects of altitude, an altitude classification test, other tests for altitude, and effects of wind, cold, and speed. Section three contains chapters on fatigue, staleness, and physical fitness, protective devices, the flight surgeon, aviation accidents, airplane dope poisoning, and civilian flying, including the medical requirements of the international congress for air navigation. There are thirty five illustrations. The book is well written, the language is terse and to the point and a strong military atmosphere pervades almost every page. One is inclined to wonder whether or not this last feature bears any relation to the relatively small number of references to the German literature which the bibliography contains. As a whole the book is a valuable contribution and undoubtedly will be widely used by all medical men who are concerned with aviation either in military or in civil life.

—D E J

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS, Mo., OCTOBER, 1926

No 1

Editor-in-Chief WARREN T VAUGHAN, M D

Richmond, Va

ASSOCIATE EDITORS

DENNIS E JACKSON, M D	CINCINNATI
PAUL G WOOLLEY, M D -	LOS ANGELES
J J R MACLEOD, M B	TORONTO
W C MACCARTY, M D -	ROCHESTER, MINN
GERALD B WEBB, M D	COLORADO SPRINGS
VICTOR C MYERS, Ph D	IOWA CITY
RUSSELL L HADEN, M D -	ROSEDALE, KAS
JOHN A. KOLMEY, M D	PHILADELPHIA
ROBERT A. KILDUFFE, M D	ATLANTIC CITY, N J
A PARKER HITCHENS, M D	MANILA, P I
DEAN LEWIS, M D - -	BALTIMORE
GEORGE R HERRMANN, M D -	NEW ORLEANS
HAROLD D CAYLOR, M D - -	ROCHESTER, MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1926 by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis Mo as Second-Class Matter

EDITORIALS

Alkalosis

ALTHOUGH the term acidosis has been in common clinical use for many years, its antithesis, alkalosis, was first employed about ten years ago. Definite proof of the clinical existence of alkalosis has, however, only recently been presented.

Ever since the administration of sodium bicarbonate found common therapeutic use, cases have occasionally been noted where its administration has given rise to disturbing clinical symptoms. It is presumably for this reason that several prominent internists have long been strongly opposed to its use in combating the acidosis of diabetes. With the introduction of the Van Slyke method of estimating the blood bicarbonate, it has been recognized that high figures for the blood CO_2 might be encountered not only after alkali administration but also following obstruction high up in the alimentary tract. Since it was originally believed that acidosis conditions remained compensated until shortly before death, there was no reason to suppose that

conditions with high bicarbonate findings should be uncompensated, i.e. show a high P_H . With the introduction of satisfactory methods of estimating the hydrogen ion concentration of the blood however it was observed that acidosis conditions became uncompensated much earlier than had been supposed. This quite naturally called attention to the opposite condition uncompensated alkalosis.

It has required considerable time to correlate the various observations bearing on this acid base balance. The studies of Hasselbalch, L. J. Henderson, Yandell Henderson, Van Slyke and their coworkers, in particular, have done much to clarify our conception of this subject. Through their work it has come to be recognized that there is a definite relation among three interdependent variables sodium bicarbonate, carbonic acid and P_H ; the determination of any two permitting the calculation of the third. Stated in another way the P_H is a function of the ratio $\frac{[HCO_3^-]}{[H^+][CO_2]}$. Anything that will raise the sodium bicarbonate or lower the carbonic acid will raise the P_H and conversely anything that will lower the sodium bicarbonate or raise the carbonic acid will lower the P_H . Several years ago Van Slyke gave us a very clear presentation of this general problem.

Alkalosis may result from either an alkali excess or CO_2 deficit. If the organism is unable to compensate for this by keeping the ratio between the bicarbonate and carbonic acid constant at about 19 to 1 we have an abnormally high P_H and a condition of uncompensated alkalosis. The condition of high bicarbonate with normal P_H is referred to as compensated alkalosis. This may result not only from compensated alkali excess but also from CO_2 excess. As a matter of fact the latter condition was one of the first to have been clearly recognized. In 1920 Scott showed that in emphysema the deficient ventilation of the blood in the lungs was followed by a compensatory retention of bicarbonate.

Conditions of uncompensated alkalosis are of the greatest interest and clinical significance, however, although where the alkalosis is due to an alkali excess it is almost invariably preceded by a condition of compensated alkalosis, i.e. high bicarbonate and normal P_H . Four types of uncompensated alkalosis have been definitely recognized clinically, namely: that following (1) sodium bicarbonate administration (2) vomiting particularly in 'high' intestinal obstruction (3) over ventilation due to fever and (4) x-ray or radium therapy. The first two of these conditions are due to alkali excess, the third is due to CO_2 deficit while in the fourth the uncompensation may occur with a perfectly normal blood bicarbonate.

Although alkalosis is much less common clinically than acidosis it is very important to recognize since the condition is much more difficult to treat than acidosis. The symptoms are rather difficult to recognize and in the past they have often been confused with acidosis thus leading to therapy that directly aggravates the condition. One case came to my attention where three different men separately made a diagnosis of acidosis on the basis of the nausea and vomiting and each prescribed the same therapy, namely gastric lavage and bicarbonate. The more prominent clinical symptoms may be given as headache, lassitude, nausea, vomiting, fever and in severe cases tetany.

Since vomiting and fever may lead to alkalosis, there exists a vicious circle, and it sometimes is difficult to recognize which is the cause and which the result. Ellis³ has well described the clinical symptoms as follows: "The patients are unduly introspective and nervous. They are irritable and complain of trifles. There is headache, nausea and vomiting, dizziness, vertigo, and light-headedness. They may complain of aching pains in the muscles and joints. There is weakness followed by absolute prostration. They become apathetic, drowsy, and are aroused with difficulty, and finally tetany and convulsions may supervene."

Toxic manifestations following the administration of sodium bicarbonate have been noted by a number of investigators in recent years, although Binger, Hastings and Neill⁴ were among the first to definitely establish the presence of an uncompensated alkalosis. In 1923 they reported observations on a case in which the P_H was raised to 7.55 (normal P_H 7.35 to 7.43) after bicarbonate administration. The following year Myers and Booher,⁵ and Kast, Myers and Schmitz⁶ presented data on 20 cases of uncompensated alkalosis, in 10 of which the alkalosis was due to bicarbonate therapy. The most striking case was a diabetic. This patient was admitted supposedly suffering from acidosis, since the urine contained a considerable amount of acetone. The small amount of alkali, however, given previous to admission, was sufficient to raise the P_H of the blood to 7.60. These workers also reported a case of cyclic vomiting in which a similar error in diagnosis had been made. Although the CO_2 content was not excessive, in this case 67 cc, the P_H was 7.52. Three months later Ellis³ reported 4 typical cases of alkalosis, in 2 of which the alkalosis was due to high-up intestinal obstruction. He has most excellently described the symptomatology of these cases. The following year Harrison and Perlzweig⁷ described a case of uremia in which alkalosis developed without the administration of sodium bicarbonate. This patient had attacks of vomiting, and they believed this afforded the best explanation of the alkalosis. The CO_2 capacity and whole blood chlorides, however, were not notably abnormal, 69 cc and 486 mg respectively, as found in cases of pyloric obstruction. The P_H was 7.6. Both Ellis³ and Myers and Booher⁵ have emphasized the fact that the kidneys may occasionally continue to secrete a strongly acid urine in certain renal conditions despite the fact that the P_H of the blood may be abnormally high. Apparently the kidneys are no longer able to excrete alkali readily.

Probably the most common causes of uncompensated alkalosis with alkali excess are the administration of sodium bicarbonate and high-up intestinal obstruction. It is apparent, however, from the cases referred to above that the etiology of uncompensated alkalosis is not always clear.

A number of cases are on record in which death, apparently from alkalosis, followed the administration of alkali. Uncompensated alkalosis following sodium-bicarbonate therapy would appear to be a condition to be guarded against, since we possess no therapeutic agent equal to bicarbonate in the treatment of acidosis. The most effective therapeutic agent in treating alkalosis is apparently ammonium chloride (Haldane⁸), the ammonia being converted to urea leaving the acidic ion. Hayden and Orr¹⁰ have shown, however, that in intestinal obstruction sodium chloride is much more effective

than ammonium chloride. The reason for this is not entirely clear, although a suitable amount of sodium chloride does restore the blood chlorides to normal and relieve the toxemia.

Although it has been known for several years that over ventilation, either voluntary or due to oxygen want, would lead to a sufficiently increased blowing off of CO₂ to disturb the bicarbonate-carbonic acid equilibrium of the blood, Koehler¹¹ was the first to give a clear and demonstration of this in acute fevers. He has reported about 10 cases of uncompensated alkalosis due to CO₂ deficit. In one case, for example, the P_H was 7.60 and the total CO₂ 47 c.c.

That deep roentgen ray therapy, particularly when applied over the chest, pelvis and abdomen, results in an acute sickness has been known for some time, although the nature of the intoxication has been obscure. Hussey¹ was the first to show that uncompensated alkali excess followed x ray exposure in rabbits. Myers and Booher confirmed this in a human subject and also noted a shift of the acid base equilibrium of the blood to the alkaline side in a case of Hodgkin's disease undergoing radium therapy. More recently Doub, Bolinger and Hartman¹² have studied the acid base balance in animals and in 150 patients treated with a modern deep therapy apparatus. A rapidly developing alkalosis and the continuation of this condition after large doses, have been shown by the determination of the P_H of the plasma and of the urine and by the use of indicators in the tissues. It is of interest that the reaction of the urine may remain alkaline for some time. Pagnez, Coste and Solomon¹⁴ observed that alkalosis developed in nine out of twelve subjects in which 500 roentgen ray units were applied to the spleen. In these cases the P_H seemed to be exclusively involved in the alkalosis.

There would appear to be no doubt then but that deep roentgen ray therapy leads to an uncompensated alkalosis. Whether this is the underlying factor or one of several factors in the intoxication following x ray therapy is not entirely clear.

The problem of uncompensated alkalosis is one deserving further careful investigation. In the meantime the clinician would do well to be quite as mindful of the occurrence and symptomatology of alkalosis as of acidosis. To be sure, the condition does not appear to be as common as acidosis but it is more difficult to recognize and treat. Unfortunately a positive diagnosis of an uncompensated alkalosis cannot be made without data on the acid base balance and this necessitates observations on the P_H of the blood as well as on the bicarbonate. In the use of alkali therapy in the treatment of acidosis it should be borne in mind that the acid base balance is occasionally quickly shifted from the acid to the alkaline side. Apparently the kidneys can no longer readily excrete alkali, since in such cases the reaction of the urine generally remains strongly acid.

REFERENCES

- ¹Van Slyke Jour Biol Chem, 1921, xlviii, 153
- ²Scott Arch Int Med, 1920, xxvi, 544
- ³Ellis Quart Jour Med, 1924, xvii, 405
- ⁴Binger Hastings and Neill Arch Int Med, 1923, xxxi, 145

- ⁵Myers and Booher Jour Biol. Chem, 1924, lxx, 699
⁶Kast, Myers and Schmitz Jour Am Med Assn, 1924, lxxxii, 1858
⁷Harrison and Perlzweig Jour Am Med Assn, 1925, lxxxiv, 671
⁸Myers and Booher Proc Soc Exper Biol Med, 1925, xxi, 513
⁹Haldane Jour Physiol, 1921, lv, 265
¹⁰Hayden and Orr Jour Am. Med Assn, 1924, lxxxii, 1515
¹¹Koehler Arch Int Med, 1923, xxxi, 590
¹²Hussey Jour Gen Physiol., 1921 22, iv, 511
¹³Doub, Bollinger and Hartman Jour Am Med Assn, 1925, lxxxv, 1299
¹⁴Pagniez, Coste and Solomon Compt rend. Soc de biol, 1925, xxi, 489

—V C M

Errata

In the article "Permanent Standards To Be Used with Benedict's Clinical Quantitative Test for Sugar in Urine" by Jeanette Allen Behre and William Muhlberg, June issue, the sentence in the twelfth line from the bottom of page 887 should read One c c of urine, 3 c c of 0.2 per cent picric acid, 0.5 c c of 5 per cent sodium hydroxide and 4 drops of 50 per cent acetone (prepared fresh each day by dilution of acetone) are added in the order named, and the tube transferred at once to a boiling water-bath and heated for from ten to fifteen minutes, cooled and the contents diluted with water to 25 c c

The legend to Chart I, Heimkamp article, page 1069, August issue, should read The full lines in this chart represent parasympathetic action and the broken line sympathetic

The Journal

LIBRARY
RECEIVED
DEC 3 1926

of U.S. Department of Agriculture

Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D. Editor in Chief
Medical Arts Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.D.
University of Toronto Toronto

Clinical Pathology

W. C. MACCARTY, M.D.
Mayo Clinic, Rochester Minn.

Biochemistry

VICTOR C. MYERS, Ph.D.
State University of Iowa Iowa City

Immunology

JOHN L. KOLMER, M.D.
University of Pennsylvania,
Philadelphia

Bacteriology

A. PARKER HITCHENS, M.D.
Bureau of Science Manila, P. I.

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

Pathology

PAUL G. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

HAROLD D. CAYLOR, M.D.
Mayo Clinic, Rochester Minn.

Experimental Medicine

RUSSELL L. HADEN, M.D.
University of Kansas Rosedale

ROBERT A. KILDUFFE, M.D.
Atlantic City N. J.

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY, 321 PL. E. BLVD. ST. LOUIS
Entered at the Post Office at St. Louis Mo. as Second Class Matter

LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment Estimates Cheerfully Furnished Prompt Service

See our advertisements, pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA PA.

[[NUMBER FOUR OF A SERIES]]

Little Glimpses

OF THE SCIENTIFIC AND RESEARCH WORK
OF PARKE, DAVIS & COMPANY



NUTRITIONAL CHEMISTRY

Centering to a large extent on a study of the vitamins, research work in nutritional chemistry has had an important bearing on general dietetics and on the study of the so-called deficiency diseases. New light has been thrown on many hitherto obscure problems.

PARKE, DAVIS & COMPANY

Home Offices and Laboratories

DETROIT, MICHIGAN

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO NOVEMBER 1926

No 2

CLINICAL AND EXPERIMENTAL

SODIUM THIOSULPHATE AND CALCIUM SALTS IN PREVENTION OF THE SEQUELAE OF ILLUMINATING GAS POISONING*

BY W H ZEIGLER † PHAR D CHARLESTON SOUTH CAROLINA

THIS report has to do with a study of the sequelae of acute illuminating gas poisoning together with an outline of a treatment for their prevention

Poisoning from this gas may be divided into acute, chronic and relapsing types The symptoms of acute poisoning are well known, but the sequelae have never been given the emphasis they should have The tendency in cases of acute poisoning is to render first aid by removing the patient from the atmosphere of the gas, by using the pulmotor or some other form of artificial respiration, by administering oxygen or oxygen plus carbon dioxide by inhalation until the patient returns to consciousness, and then to feel that we have done our duty To quote Henderson and Haggard,¹ who are authorities on illuminating gas poisoning 'Within a few hours after profound but not fatal poisoning from carbon monoxid no trace of the gas is found in the blood, and yet for days, months, or even for life, structural degenerations usually either nervous or cardiac may continue' They also give as their opinion that 'during the first hour about half the amount previously absorbed is eliminated' and suggest that 'the post gassing period of continued asphyxia is of critical importance in inducing structural degeneration and functional impairment'

Kurlander sums up the pathologic changes as follows "There are marked degenerative changes in the muscles and, in most instances small scattered hemorrhages and intense hyperemia of the organs The most important nerve lesions are in the brain"

Read in abstract before the Medical Society of South Carolina January 2nd 1926
Received for publication, April 16 1926
†Professor of Pharmacology Medical College of the State of South Carolina

The sequelae of acute poisoning from this gas are summed up by McConnell and Spiller³ as follows "Pneumonia, cardiac palpitation, localized hyperemia, gastrointestinal disturbances, transient glycosuria, cutaneous eruptions, localized edema and gangrene. In the nervous system, paralysis of the central or peripheral type. Persistent headache is complained of and mental changes take place, often only mild hallucinations but more commonly distinct confusional insanities. Relapsing carbon monoxid poisoning is a term used to indicate the condition in those who had apparently recovered from the initial effects of the poison only to develop, after a period of fair health, a grave type of symptoms leading to death."

Carbon monoxid is the toxic agent in illuminating gas although it is the opinion of Henderson and Haggard¹ that 25 per cent of its toxicity is due to some other substance. We have been taught that carbon monoxid combines with hemoglobin to form a strong combination which is very difficult to break up. The opinion of authorities today is that while it combines with the hemoglobin, the combination is easily broken up. If this is true, how can we explain the sequelae and the delayed symptoms? It has been suggested, as an explanation, that in carbon monoxid poisoning "by imperfect elimination injurious metabolic substances accumulate and produce profound effects upon the cerebral vessels."

It was not the intention of the author of this investigation to seek for causes but to attempt to find some method of preventing the sequelae so often seen in this class of poisoning. With this idea in view, a series of experiments were planned in which the animals used were dogs. After a number of preliminary experiments with different percentages of the gas, an amount was found which would produce mental and other delayed symptoms in the animal.

EXPERIMENTS

Dogs were the animals used throughout the investigation. The animals were placed in a specially constructed chamber provided with a glass door through which they could be watched. City illuminating gas was delivered through a tube connected to a box. A very delicate gas meter was used to register accurately the amount of the gas necessary to give a 2 per cent atmosphere.

PROTOCOL

Two per cent illuminating gas followed by the intravenous injection of 5 cc of a 2 per cent solution of sodium thiosulphate per kilo and the subcutaneous injection of 2 cc of a 1 per cent solution of calcium lactate per kilo.

Dog, 6.8 kg	July 23, 1925	10 45 A.M.	2 per cent illuminating gas
		10 50 A.M.	Discomfort with excessive salivation
		10 55 A.M.	Struggles and whines, falls
		11 00 A.M.	Down
		11 02 A.M.	Vomits
		11 10 A.M.	Convulsions, skin deep red
		11 20 A.M.	Respiration shallow and rapid
		11 31 A.M.	Removed from chamber
		11 35 A.M.	5 cc 2 per cent solution per kg of sodium thiosulphate given intravenously. Also 2 cc 1 per cent calcium lactate per kg injected subcutaneously

	11 40 A.M	Evidence of recovery
	12 10 P.M	Gets up and walks around. Staggers and seems confused
	12 36 P.M	2 c.c. 1 per cent calcium lactate per kg. subcutane only
July 24,	1 00 P.M	Walks around
August 21,		Fully recovered
		Alive

TABLE I
GAS 2 PER CENT WITHOUT TREATMENT

NUMBER	IN CHAMBER	CONDITION ON REMOVAL	
1	35 min	Gasping	Died before treatment could be given
2	25 min	Gasping	Died before treatment could be given
3	25 min	Gasping	Died before treatment could be given
4	26 min.	Not breathing	Died before treatment could be given
5	30 min	Breathing	Died before treatment could be given
6	40 min	Gasping	Died before treatment could be given
7	38 min	Gasping	Died before treatment could be given
8	25 min	Gasping	Died before treatment could be given
9	30 min.	Not breathing	Died before treatment could be given
10	28 min	Gasping	Died before treatment could be given
11	25 min	In convulsion	Died before treatment could be given
12	30 min	Gasping	Died before treatment could be given

in the chamber. The illuminating gas used contained about 25.2 per cent of carbon monoxide. One tenth of one per cent of carbon monoxide will prove fatal in time, and 1 per cent will cause death in a few minutes. An atmosphere of 2 per cent illuminating gas was decided upon because of this and also due to the fact that after a number of experiments with varying amounts of the gas it was found to be fatal in the majority of cases. Protocol will show how quickly the first symptoms were produced. These usually came in less than five minutes. The animals were allowed to remain in the chamber until the respiration had almost ceased. As seen by the records, this varied. The shortest time being twenty seven minutes and the longest fifty minutes. Table I will show by the number of deaths which occurred before treatment could be given, how close the amount of gas used and the time of exposure approached the fatal dose.

After the proper amount of gas was determined, together with the time the animals were to be subjected to its influence the next step was to find some substance which would chemically change or combine with the carbon monoxide of the illuminating gas. Since it is quite evident that "this very poisonous gas penetrates rapidly into the cells the success of any treatment would depend upon the rapidity with which it could be detoxicated."

Meyer and Gottlieb⁴ give as their opinion "When the combination formed by the poison and the protoplasm or more correctly the reacting constituent thereof, is reversible with difficulty or not at all (for instance on account of its complete insolubility), it is quite clear that even an adequate antidote which is able to combine with the poison cannot reverse the toxic reaction. In such cases however it may be possible to repair the protoplasm by replacing such of its constituents as have combined with the toxic agent. This is actually what occurs in the antagonistic action of lime salts in oxalate poisoning. When on the other hand the toxic reaction is readily reversible as for example in chloral

or chloroform poisoning, a substance which possesses an avidity for the toxic substance equal to or greater than that of the cell constituents can attract the toxic substance to itself and thus overcome the poisoning of the cell "

In the case reported by McConnell and Spiller,³ there was a marked calcification of the capillaries of the brain, being more pronounced in the segments of the lenticular nucleus. They suggest that this condition is due to the calcium salts of the blood being thrown out of solution, caused by utilization of the proteids which held them in solution or suspension. Since sodium thiosulphate had been used with success in poisoning by lead tetraethyl, the nitrils, and cyanides, it was thought that perhaps this chemical would distoxicate or break up the combination of the carbon monoxid with the hemoglobin more rapidly.



Fig 1—Photograph of one of the dogs on the twenty-first day, showing delayed symptoms

Calcium chloride was also injected subcutaneously at regular intervals with the hope of replacing this element and at the same time increasing the coagulability of the blood.

After a number of preliminary experiments in which measured amounts of sodium thiosulphate were injected intravenously and calcium chloride subcutaneously, the following procedure was found to be most effective. Immediately upon removing the animal from the chamber, artificial respiration was administered. If this was successful, 5 c c of a 2 per cent solution of sodium thiosulphate per kilo was injected into the vena saphena parva. At the same time, 2 c c of a 1 per cent solution of calcium chloride per kilo was injected subcutaneously. As seen by Table II, the recovery was not only more rapid, but, of the 14 dogs reported, only 1 developed mental symptoms and only 1 died. As seen by Table III, 12 dogs were subjected to the same percentage of gas over practically the same period of time without treatment. Two of the animals showed no mental symptoms, 2 recovered, 1 of these showing on the sixth day a weakness in the hind legs, and the other 8 after developing marked mental and other symptoms finally died.

TABLE II
GAS 2 PER CENT WITH TREATMENT

NUMBER	IN CHAMBER	1ST SIGN OF RECOVERY	MENTAL SYMPTOMS	
1	33 min	9 min	None	Recovered
2	30 min	6 min	None	Recovered
3	31 min	5 min	None	Recovered
4	30 min	8 min	None	Recovered
5	36 min	12 min	None	Recovered
6	27 min	8 min	None	Recovered
7	43 min	11 min	None	Recovered
8	42 min	3 min	None	Recovered
9	41 min	13 min	None	Recovered
10	34 min	5 min	13th day drowsy	18th day died
11	28 min	17 min	None	Recovered
12	30 min	10 min	None	Recovered
13	33 min	5 min	None	Recovered
14	46 min	14 min	None	Recovered

TABLE III
GAS 2 PER CENT WITHOUT TREATMENT

NUMBER	IN CHAMBER	1ST SIGN OF RECOVERY	MENTAL SYMPTOMS	
1	46 min	36 min	14th day drowsy irresponsive	28th day died
2	30 min	15 min	6th day paralysis of hind legs	8th day died
3	30 min	10 min	6th day weak in hind legs	Recovered
4	35 min	23 min	7th day weak and drowsy	10th day died
5	30 min	30 min	None	Recovered
6	40 min	12 min	16th day depressed with choreiform movements of muscles	20th day died
7	46 min	10 min	21st day weak and drowsy	22nd day died
8	39 min	36 min	3rd 8th day weak, nervous disturbance of equilibrium	9th day died
9	50 min	25 min	2nd 25th day depressed irritable disturbance of equilibrium	26th day died
10	30 min	18 min	8th day had convulsion	37th day died
11	30 min	12 min	None	Recovered
12	31 min	20 min	40th day drowsy	Recovered

Protocol gives an outline of the symptoms and treatment of the animals in this series of experiments

The 38 dogs reported in the three tabulations were grouped according to results and not in sequence

AN OUTLINE FOR TREATMENT IN HUMAN SUBJECTS

Up to the time of the preliminary report of this investigation the author has not had the opportunity of demonstrating upon human subjects the treatment which was found effective for animals. I would suggest, however, as a result of these experiments, the following treatment. First remove the patient from the atmosphere of the illuminating gas. Administer artificial respiration if necessary and, as soon as possible, inject intravenously 5 c.c. of a 2 per cent solution of sodium thiosulphate for every 25 pounds of body weight. An individual weighing 150 pounds would require 30 c.c. of the solution. This amount would contain about 10 grains of the thiosulphate of soda. Kuhn and Reese* in a study of sodium thiosulphate in the treatment of metallic intoxication injected intravenously amounts of 15 grains over a period of fourteen days without any bad effects.

Sodium thiosulphate is oxidized in the body to sodium sulphate and a molecule of sulphur is liberated

The next step in the treatment would be the subcutaneous injection of 5 c c of a 4 per cent solution of calcium lactate for each 25 pounds body weight. After consciousness has returned, calcium lactate should be given in a daily dose of 1 gram and continued for several days.

The following is a summary of a case which occurred in our city while this investigation was in progress and will serve to illustrate the sequelae of illuminating gas poisoning. The treatment consisted in the administration of chloral hydrate and sodium bromide.

CASE HISTORY

M F, white, female, aged four years. On Sunday, January 9, patient was found in bathroom unconscious, asphyxiated with illuminating gas. Child was removed, resuscitated and brought to hospital. Regained normally and was sent home on Monday with apparently no ill effect. In the afternoon of the same day the child became very nervous and restless. The mother fearing convulsions returned the child to the hospital. General appearance of child was that of anxiety and restlessness. Physical examination was negative except for dilated pupils and hyperactive reflexes. Urinalysis negative. Temperature normal. W B C 22,080. Polymorphonuclears 85.

Jan 11 Child vomited. Rested well.

Jan 12 Patient had several generalized convulsions in afternoon.

Jan 13 Temperature 100°. Rather somnolent.

Jan 14 No change.

Jan 15 Urinalysis Acetone + + + +, others negative. Blood 10,000. Polymorphonuclears 41. L L 44, etc. Tendency for child not to move upper extremities.

Jan 16 Patient has paralysis of left upper extremities. Apparent inability to perceive objects although she does distinguish light. Pulse 72. Spinal fluid not under pressure. Analysis normal. X ray shows no evidence of cranial injury.

Jan 17 Patient had two convulsions in left side. Can move left hand.

Jan 18 Has control of muscles. Retardation of muscular movement.

Jan 19 Temperature normal, urinalysis normal. Improving.

Jan 20 Improved.

Jan 23 Patient discharged, apparently normal.

CONCLUSIONS

1 It is possible to produce in dogs the sequelae of carbon monoxid poisoning such as mental disturbances, general weakness, skin eruptions, etc.

2 It is possible to prevent these symptoms by the intravenous injection of sodium thiosulphate and the subcutaneous injection of calcium salts.

The author wishes to express his appreciation for the valuable assistance rendered by Dr H B Holmes and Mr E C Hood.

REFERENCES

- ¹Henderson and Haggard Jour of Pharmacol and Exper Therap, 1921, v, 11
- ²Kurlander, J J Jour Am Med Assn, 1924, lxxxiii, 271
- ³McConnell, J W, and Spiller, W G Jour Am Med Assn, 1912, lxx, 2122
- ⁴Meyer and Gottlieb Pharmacol Clin Exper, 569
- ⁵Kuhn, H A, and Reese H H Jour Am Med Assn, 1925, lxxxv, 1804

THE EFFECT OF HYPODERMIC INSULIN ON THE FASTING BLOOD SUGAR IN NORMAL AND DIABETIC SUBJECTS IN RELATION TO PERCENTAGE NORMAL WEIGHT*

BY WALTER M. BARTLETT, M.D., BOSTON, MASS

IT has been noted by various observers that the effect of hypodermic injections of insulin as measured by the fall in blood sugar in mg per 100 cc is largely dependent upon the level of the blood sugar at the time of injection.¹ In other words, it is common experience that insulin produces a more marked effect when the initial blood sugar level is high. In view of this fact it was thought that if insulin has a more specific blood sugar lowering effect in diabetic persons that it might offer a better diagnostic procedure than the usual glucose tolerance test. Consequently the blood sugar effect after hypodermic insulin has been studied in a series of cases including normal and diabetic subjects.

It was at first noted that the blood sugar effect was influenced by the relation of body weight to normal. This has offered the best method of classifying the cases.

TECHNIC

The patient's body weight and height were taken in the morning, the patient's fasting blood sugar determined and 10 units of U 20 Insulin Lilly (lletin) was given subcutaneously. Breakfast was omitted on the morning of the test, and subsequent blood sugar determinations were made at one hour and a half and three hours and a half after insulin. Blood sugar determinations were done by the Folin Wu method. Percentage normal weight was calculated from the tables of the Association of Life Insurance Directors and Actuarial Society of America.² The normal subjects referred to are in part healthy young adults and in part convalescent ward cases in whom we had no reason to expect any disturbance in carbohydrate metabolism.

DATA

Table I shows the effect of 10 units of insulin given hypodermically on the fasting blood sugar of ten normal subjects whose body weight is within 4 per cent of normal. The maximum fall in blood sugar occurred in each case three hours and a half after injection, and the average fall was 28 mg per 100 cc of blood.

Table II shows the level of blood sugar before and after insulin in eight obese subjects whose body weight varies from 18 to 39 per cent above normal. Here the maximum fall in blood sugar occurred at the end of one hour and a half, and, although the fasting blood sugar was higher than in normal subjects of normal weight, the fall in blood sugar averaged 28 mg as in Table I.

*From the Thorndike Memorial Laboratory of the Boston City Hospital Boston, Mass.
Received for publication April 13 1926

TABLE III
EFFECT OF HYPODERMIC INSULIN IN UNDERWEIGHT NORMAL SUBJECTS

[illegible]

TABLE IV
EFFECT OF HYPODERMIC INSULIN IN DIABETIC SUBJECTS UNDER CONTROL

CASE NO	INITIALS	AGE	WEIGHT	HEIGHT	% NORMAL WEIGHT	INSULIN	BLOOD SUGAR IN MG PER 100 C C				MAXIMUM FALL	HR.
							Fa mg	15 Hr	mg	35 Hr		
Patients above Normal Weight												
1	M G	02	180	5 10	+ 5	10	160	128	99	51	35	
2	C R	53	172	5 8"	+ 6	10	143	136	82	61	35	
3	M M	48	168	5 3"	+17	10	120	120	30	30	35	
4	V S	38	165	5 6	+12	10	130	116	78	52	35	
										Average	50	
Patients below Normal Weight												
5	G R	49	123	5 7"	-19	10	128	62	66	60	15	
6	J O	8	47	4 1"	-15	10	120	42	55	78	15	
7	R B	5	40	3 7"	-5	10	122	46	76	61	15	
8	W R	10	59	4 9"	-20	10	116	52	69	64	15	
9	J T	36	117	5 6"	-25	10	132	62	84	70	15	
										Average	70	

TABLE I
DATA OF 1925 EPIDEMIC

DATE	GUINEA PIG	LYMPH NODES INVOLVED	ABSCESSSES		RESULTS OF CULTURES	
			Number	Size, cm	Glucose brain broth (primary culture)	Blood agar (primary culture)
1-20	306	Cervical	1	3.0	Streptococci	Hemolytic streptococci
1-23	307	Submaxillary	1	2.5	Streptococci	Hemolytic streptococci
1-27	308	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
1-27	309	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
1-27	310	Submaxillary	1	1.5	Streptococci	Hemolytic streptococci
2-3	311	Submaxillary	2	1.0	Streptococci	Hemolytic streptococci
2-3	312	Inguinal	1	2.0	Streptococci	Hemolytic streptococci
2-3	313	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2-5	326	Right preaural and submaxillary	1 2	0.5 0.5	Streptococci	Hemolytic streptococci
2-5	327	Left preaural and submaxillary	1 2	1.0 1.0	Streptococci	Hemolytic streptococci
2-5	328	Submaxillary	4	0.5	Streptococci	Hemolytic streptococci
2-5	329	Submaxillary	1	2.0	Streptococci	Hemolytic streptococci
2-5	330	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2-5	331	Inguinal	1	2.0	Streptococci and <i>Bacillus coli</i>	Hemolytic streptococci and <i>Bacillus coli</i>
2-5	332	Parotid	1	0.5	Streptococci	Hemolytic streptococci
2-5	333	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2-6	334	Cervical	1	1.5	Streptococci	Hemolytic streptococci
2-6	335	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2-6	336	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2-6	337	Parotid	1	1.5	Streptococci	Hemolytic streptococci
2-6	338	Submaxillary and each preaural	1 2	0.5 0.5	Streptococci	Hemolytic streptococci
2-6	339	Submaxillary	1	1.5	Streptococci and <i>Bacillus subtilis</i>	Not cultured
2-6	340	Right preaural and submaxillary	1 1	0.5 0.5	Streptococci	Hemolytic streptococci
2-6	341	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2-6	342	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2-6	343	Right preaural and submaxillary	1 1	0.5 0.5	Streptococci	Hemolytic streptococci
2-6	344	Cervical	1	1.5	Streptococci	Hemolytic streptococci
2-6	345	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2-9	358	Submaxillary	2	0.5	Streptococci	Hemolytic streptococci
2-9	359	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2-9	360	Cervical	2	1.0	Streptococci	Hemolytic streptococci
2-9	361	Cervical	3	1.0	Streptococci	Hemolytic streptococci
2-9	362	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2-9	363	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2-11	372	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2-11	373	Submaxillary	2	0.3	Streptococci	Hemolytic streptococci
2-11	374	Submaxillary	2	1.5	Streptococci	Hemolytic streptococci
2-11	375	Submaxillary	1	2.0	Streptococci	Hemolytic streptococci
2-11	376	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2-11	377	Cervical (?)	?	?	No growth (node only inflamed)	
2-11	378	Cervical and right preaural	1 1	1.0 0.5	Streptococci	Hemolytic streptococci
2-11	379	Right preaural	1	1.5	Streptococci	Hemolytic streptococci
2-11	380	Right preaural	1	0.5	Streptococci	Hemolytic streptococci
2-11	381	Submaxillary and left preaural	1 1	0.5 0.5	Streptococci	Hemolytic streptococci
2-11	382	Submaxillary	3	1.5	Streptococci	Hemolytic streptococci
2-11	383	Left preaural	1	1.0	Streptococci	Hemolytic streptococci
2-18	393	Left preaural	1	1.5	Streptococci	Hemolytic streptococci
2-18	394	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2-21	395	Submaxillary	1	0.5	No growth	
2-21	396	Left preaural	1	1.0	Streptococci	Hemolytic streptococci

TABLE I—CONT'D

DATE	GUINEA PIG	LYMPH NODES INVOLVED	ABSCESES		RESULTS OF CULTURES	
			Number	Size, cm	Glucose brain broth (primary culture)	Blood agar (primary culture)
2-21	397	Cervical	1	1.5	Streptococci	Hemolytic streptococci
2-21	398	Left preaural	1	1.0	Streptococci	Hemolytic streptococci
2-21	399	Submaxillary	1			
		Cervical	1	1.0	Streptococci	Hemolytic streptococci
2-21	400	Cervical	1	0.5	Streptococci	Hemolytic streptococci
2-21	601	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2-21	602	Submaxillary	2	0.5	No growth	
2-14	314*	Cervical	2	1.0	Streptococci and <i>Bacillus coli</i>	Hemolytic streptococci and <i>Bacillus coli</i>
		Preaural	1			
2-14	317*	Submaxillary	4	2.0	Streptococci	Hemolytic streptococci

*Died

TABLE II
DATA OF 1926 EPIDEMIC

DATE	GUINEA PIG	LYMPH NODES INVOLVED	ABSCESES		RESULTS OF CULTURES	
			Number	Size, cm	Glucose brain broth (primary culture)	Blood agar (primary culture)
2-22	700	Preaural	2	1.0	Streptococci	Hemolytic streptococci
2-23	701	Submaxillary	1	1.5	Streptococci	Hemolytic streptococci
2-23	702	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2-23	703	Cervical	2	2.0	Streptococci	Hemolytic streptococci
2-23	704	Cervical	1	0.5	Streptococci	Hemolytic streptococci
2-23	705	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2-23	706	Cervical	1	2.0	Streptococci	Hemolytic streptococci

to twenty four hours, the resulting growth was examined and plated on blood agar in order to observe hemolysis and to determine the purity of the culture

A total of eighty nine abscesses were found in the fifty eight guinea pigs in the 1925 series, of which forty five involved the submaxillary lymph nodes, twenty six the cervical nodes, fourteen the preaural, two the parotid, and two the inguinal

Pure cultures of hemolytic streptococci were obtained from the lesions of fifty one of the fifty eight guinea pigs no growth was obtained in three, and in four, mixed cultures showing association or contamination with *Bacillus coli* twice, *Bacillus subtilis*, once and *Staphylococcus albus* once

SYMPTOMS AND LESIONS OF SPONTANEOUS CASES

The disease is symptomless except for the swelling, which may be visible or recognized only by palpation. The general health of the animals, with one or two exceptions, has not been impaired to any extent. The process is essentially one of the formation of "cold" abscesses involving the lymph nodes without evidence of reaction in the surrounding tissues except for the formation of a rather indefinite limiting membrane. Approximately half of the animals show involvement of the submaxillary lymph nodes, in one fourth the preaural nodes are affected, and in the remaining fourth the lesions are divided among the cervical, parotid, and inguinal regions. The abscesses in the submaxillary and cervical regions may be single or multiple, varying from 3 mm to 2.5 cm in diameter. Multiple abscesses are seldom encountered in the other sites.

The pus contained in the abscesses is always thick homogeneous, non odorless and yellowish white, apparently it never becomes cheesy or calcareous

TABLE IV
INOCULATION WITH VARIOUS STRAINS OF STREPTOCOCCI

DATE 1925	ANIMALS INJECTED	INFECTION MATERIAL		INJECTION GLUCOSE BRAIN BROTH		RESULT OF INJECTION
		Source	Age of culturo	Amount c.c.	Mode	
2-23	Guinea pig 603	Guinea pig 396 ous, left preaural node	2 days	0.5	Subcutaneous	Local inflammation and swelling 3-1-25 Large abscess at point of injection 3-17-25 (Animal killed) Abscess contained character istic pus Pure culture of hemolytic streptococci from abscess Culture of heart blood sterile
2-23	Guinea pig 604	Guinea pig 399 ous, submaxillary node	2 days	0.5	Intrapentoneal	Death 2-26-25 Generalized, adhesive peritonitis with large amount thick, purulent exudate on all peritoneal surfaces Culture of perit oneal fluid and heart blood yielded pure cultures hemolytic strepto cocci
2-23	Rabbit 605	Guinea pig 400 ous, cervical node	2 days	1.0	Subcutaneous	Local swelling and abscess formation 2-27-25 Death 3-3 Large area necrosis and abscess formation, generalized purulent perit onitis Pure cultures hemolytic streptococci from abscess, heart blood and peritoneal fluid
2-23	Rabbit 606	Guinea pig 601 ous cervical node	2 days	0.5	Intravenous	Death 2-26-25 Pericarditis, hemorrhagic epicarditis, generalized adhesive peritonitis with large quantities of purulent exudate on peritoneal surfaces, pleurisy Pure cultures from peritoneal fluid, heart blood and pleural fluid
3-5	Rabbit 610	Rabbit 606	6 days	0.1	Intravenous	Animal remained well
3-5	Rabbit 611	Rabbit 606	6 days	0.2	Intrapentoneal	Death 3-8-25 Generalized peritonitis, serofibrinous pleurisy, peri carditis, and small abscess at point of entrance of needle Pure cultures hemolytic streptococci from abscess, peritoneal fluid, pleu ral fluid and heart blood
3-5	Guinea pig 612	Rabbit 606	6 days	0.5	Intrapentoneal	Animal remained well

TABLE IV—Continued

DATE	ANIMALS INJECTED	INJECTION MATERIAL		INJECTION GLUCOSE RELAY DROTH		RESULT OF INJECTION
		Source	Age of culture	Amount c.c.	Mode	
3-5	Guinea pig 613	Rabbit 606	0 days	1.0	Subcutaneous	Local swelling, and abscess formation 3-12-26. Large abscess 2 cm diameter 3-17 (Animal killed). Abscess contained characteristic pus. Pure cultures of hemolytic streptococci from abscess heart blood sterile.
3-18	Rabbit 616	Guinea pig 368 Artificially infected abscess	24 hours	0.5	Intravenous	Animal remained well.
3-18	Guinea pig 617	Guinea pig 368 Artificially infected abscess	24 hours	0.5	Subcutaneous	Local swelling, and abscess formation 3-20-26. Death 3-30. Large abscess at point of injection left precaral lymph node also involved. Pure cultures hemolytic streptococci from abscess, heart blood and node.
3-18	Guinea pig 618	Guinea pig 368 Artificially infected abscess	24 hours	0.5	Intraperitoneal	Death 3-22-26. Extensive subcutaneous inflammation and beginning abscess formation along abdominal wall. Generalized peritonitis. Pure cultures hemolytic streptococci from heart blood, spinal and peritoneal fluid.
3-18	Rabbit 619	Guinea pig 368 Spleen	24 hours	0.5	Intravenous	Animal remained well.
3-18	Guinea pig 620	Guinea pig 368 Spleen	24 hours	0.5	Subcutaneous	Death 3-24-26. Extensive subcutaneous abscess formation and no abscess. Pure culture hemolytic streptococci from subcutaneous tissue and heart blood.
3-18	Guinea pig 621	Guinea pig 368 Spleen	24 hours	0.5	Intraperitoneal	Death 3-25-26. Subcutaneous sero-anguineous exudate. Large inflammatory mass in right inguinal region with beginning abscess formation. Pure culture hemolytic streptococci from abscess and peritoneal fluid.

TABLE VI

RESULTS OF INOCULATING GUINEA PIGS WITH VARIOUS FILTRATES AND PUS EMULSION

GUINEA PIG	INJECTION 2-9-25			RESULT
	Amount c c	Material	Mode	
346	0.2	Filtrate 1	Subcutaneous	Animal remained well
347	1.0	Filtrate 1	Subcutaneous	Animal remained well
348	0.1	Filtrate 1	Intraperitoneal	Animal remained well
349	0.5	Filtrate 1	Intraperitoneal	Animal remained well
350	0.2	Filtrate 2	Subcutaneous	Animal remained well
351	1.0	Filtrate 2	Subcutaneous	Animal remained well
352	0.1	Filtrate 2	Intraperitoneal	Animal remained well
353	1.0	Filtrate 2	Intraperitoneal	Animal remained well
354	1.0	Filtrate 3	Subcutaneous	Animal remained well
355	2.0	Filtrate 3	Subcutaneous	Animal remained well
356	0.2	Filtrate 3	Intraperitoneal	Animal remained well
357	1.0	Filtrate 3	Intraperitoneal	Animal remained well
364	0.5	Filtrate 4	Subcutaneous	Animal remained well
365	1.0	Filtrate 4	Subcutaneous	Animal remained well
366	0.2	Filtrate 4	Intraperitoneal	Animal remained well
367	0.5	Filtrate 4	Intraperitoneal	Animal remained well
368	0.2	Pus emulsion	Subcutaneous	2-11-25 abscess 2 cm diameter at point of injection 2-14-25 abscess opened spontaneously and draining 2-27-25 nearly healed See necropsy notes
369	0.5	Pus emulsion	Subcutaneous	2-11-25 swelling at point of injection 2-13-25 died See necropsy notes
370	0.1	Pus emulsion	Intraperitoneal	2-14-25 animal died See necropsy notes
371	0.2	Pus emulsion	Intraperitoneal	2-11-25 animal died See necropsy notes

Pathogenicity of Old Cultures—On April 10, 1925, guinea pigs 625, 627 and 629 and rabbits 626, 628 and 630 were injected intraperitoneally with 1 c c of old glucose-brain-broth cultures obtained from guinea pigs 326, 327 and rabbit 392, respectively, the first two of these cultures were from spontaneous cases (Table I) and the last was isolated from an artificially infected animal (Table III). These strains were sixty-four and fifty-two days old, respectively, at the time of this experiment and had been kept in glucose-brain-broth at room temperature. Strains 326 and 327 were still virulent for guinea pigs and rabbits, strain 392 was avirulent.

Studies relating to the serology, immunology and production of hemolysin by the infecting streptococcus in this disease are being made and may form the basis for a future paper.

DISCUSSION

The condition described here is not to be confused with pseudotuberculosis of guinea pigs noted by several writers and caused by an organism designated as *Streptococcus pseudotuberculosis rodentium* (*Coincybacterium rodentium*), a bacillus with rounded ends, short, thick, and gram-negative, having a tendency to form long chains in tissue as well as in artificial mediums. The lesions of so-called pseudotuberculosis of guinea pigs are usually found in the liver, spleen and intestine, and may be cascated, in contradistinction to the lesions of streptococcal lymphadenitis.

One of the most striking features in this study was the innocuousness of natural infection as compared with artificial infection. Few spontaneously in

Filtrate 1—Two tubes of pancreatic digest broth were inoculated with glucose brain cultures of strains of hemolytic streptococci isolated from guinea pigs 307 and 308. These were incubated at 37° C for four days, an excellent growth of streptococci occurred and the broth culture was passed through a sterile Berkefeld filter, and its sterility was proved by an attempt to culture it. This was used to inject guinea pigs 346, 347, 348 and 349 (Table VI).

Filtrate 2—This was prepared the same as *Filtrate 1* except that the organisms used were strains picked from blood agar cultures isolated from guinea pigs 309, 310 and 311. The filtrate from these cultures was sterile and used to inject guinea pigs 350, 351, 352 and 353 (Table VI).

Filtrate 3—Glucose brain broth tubes were inoculated with strains from guinea pigs 301, 308, 309, 310 and 311. Sterile filtrates were prepared as before, and used to inject guinea pigs 354, 355, 356 and 357 (Table VI).

Filtrate 4—Three cubic centimeters of pus were collected in a sterile manner from the abscesses of spontaneously infected guinea pigs 329 and 360. This pus was mixed with 15 cc of sterile sodium chloride solution then filtered through a sterile Berkefeld filter and cultured to determine its sterility. It was injected into guinea pigs 364, 365, 366 and 367 (Table VI).

Pus emulsion—The residue remaining on the sterile Berkefeld candle used in preparing *Filtrate 4* was washed off with sterile sodium chloride solution and used to inject guinea pigs 368, 369, 370 and 371 (Table VI).

Necropsy Notes on Guinea Pigs Injected with Pus Emulsion—Guinea pig 371 died February 11, 1925. Diffuse abscesses had formed about the point of injection between the skin and abdominal wall, and general peritonitis had developed. Cultures of the peritoneal exudate and heart blood yielded pure cultures of hemolytic streptococci.

Guinea pig 369 died February 13, 1925. Abscesses had begun to form at the point of injection, and serosanguineous infiltration had taken place in the subcutaneous tissue along the entire left side of the abdominal and thoracic walls. Cultures of the subcutaneous exudate and heart blood yielded pure cultures of hemolytic streptococci.

Guinea pig 371 died February 14, 1925. General peritonitis, exudative and adhesive, had set in. Cultures of the peritoneal exudate and heart blood yielded pure cultures of hemolytic streptococci.

Guinea pig 368 developed an abscess (Table VI) which opened and drained until February 27, 1925, when it was almost entirely healed. The animal was killed March 17. Slight inflammation was noted at the point of injection, a small abscess 0.5 cm in diameter behind the right scapula, and three abscesses in the spleen, each 1 cm in diameter. Both precrural lymph nodes were inflamed and swollen. Cultures from the postscapular abscess and both precrural lymph nodes yielded pure cultures of hemolytic streptococci, culture of the heart blood was negative.

These experiments indicate that no substance such as a toxin is elaborated either by culture of the hemolytic streptococci in vitro or by the organism in vivo, that is virulent for guinea pigs. The infectious nature of the purulent exudate is noted, however, in the experiment with the last series of guinea pigs in which three succumbed and one developed lesions like those in cases of spontaneous infection.

TABLE V
RESULT OF EXPOSING HEALTHY ANIMALS TO NATURALLY INFECTED ANIMALS

GROUP	GUINEA PIG SEX	CONDITION AT BEGINNING OF EXPERIMENT, 2-5-25	RESULT OF EXPOSURE
1	314, F	Spontaneously infected Two large abscesses in cervical region Not discharging	Death 2-14-25 (Table I)
	315, F	Normal, no sign of infection	Animal exposed for nine days only No evidence of infection after two months
	316, M	Normal, no sign of infection	Animal exposed for nine days only No evidence of infection after two months
2	317, M	Spontaneously infected Four abscesses in submaxillary region and one in left precaral Not discharging	Death 2-12-25 (Table I)
	318, F	Normal, no sign of infection	Animal exposed for seven days only No evidence of infection after two months
	319, M	Normal, no sign of infection	Animal exposed for seven days only No evidence of infection after two months
3	320, F	Spontaneously infected Small discharging abscess in sub maxillary region	Abscess continued to drain until entirely healed 3-5-25
	321, F	Normal, no sign of infection	Animal exposed for one month No evidence of infection after two months
	322, M	Normal, no sign of infection	Animal exposed for one month No evidence of infection after two months
4	323, M	Spontaneously infected Small discharging abscess in sub maxillary region	Abscess continued to drain for twelve days and was then entirely healed
	324, F	Normal, no sign of infection	No evidence of infection after two months
	325, M	Normal, no sign of infection	No evidence of infection after two months

Attempted Infection by Way of the Conjunctiva—March 18, 1925, one drop of twenty-four-hour cultures of streptococci isolated from guinea pigs 388, 603 and 613 was applied to the conjunctiva of guinea pigs 622, 623 and 624, respectively. Conjunctivitis did not develop in the inoculated eyes, and the animals remained well for one month. Necropsy at this time revealed no evidence of streptococcus infection and all cultures were sterile.

Attempted Infection by Contact—An experiment was undertaken to determine whether or not infection would readily occur through contact of healthy animals with naturally infected ones (Table V). Two of the naturally infected guinea pigs, 314 and 317, died seven and nine days, respectively, after the experiment was begun. These are the only two spontaneously infected animals that have died under my observation, and it is not certain that the streptococcus infection itself was the only factor. The other two spontaneously infected animals in this experiment recovered entirely after their abscesses had drained for several weeks. None of the exposed uninfected animals gave any evidence of having acquired the infection after two months' observation and were found to be normal at necropsy.

Experiments with Regard to Toxin and Filtrable Virus—The object of this experiment was to determine whether any toxin or filtrable substance was elaborated by the infecting streptococci or was contained in the abscess material which would produce lesions similar to those in spontaneously infected animals. For this experiment, four filtrates and one control emulsion of the pus from an abscess were prepared.

feeted animals die, as the lesions are prone to rupture eventually and then heal. The epidemic nature of the infection and its apparently seasonal occurrence are characteristic of some other streptococcal infections in man and animals. The prevalence of the infection in female guinea pigs as compared to males in the ratio of about 8 to 1 would suggest infection by way of the genitalia, but this would hardly account for the apparent predilection of the organisms for the superficial lymph nodes.

In attempting to find the source of the infection, a careful check was made of all new guinea pigs received and it was found that infected animals had been received from several widely separated points. The possibility of infection from laboratory animals used for injection with various cultures of streptococci from human sources is further discounted by the fact that the reserve guinea pigs are held in stock pens in a building removed from the laboratory and are never in contact with used laboratory animals until placed on experiment. The disease, then, must be considered a natural affection.

The invasive powers of the infecting streptococci in artificial infection are illustrated by the fact that peritonitis was caused in one animal by intravenous inoculation (Rabbit 606, Table IV) and in another by subcutaneous inoculation (Rabbit 605 Table V).

Finally, the infection somewhat resembles a disease of horses commonly known as strangles (adenitis eorum) due to *Streptococcus equi* (Schutz), and which is characterized by inflammation of the nasal mucosa and suppuration of the regional lymph nodes. The equine disease, however, is an acute process with marked general disturbance and a mortality of from 2 to 5 per cent.

SUMMARY

A description is given of an epidemic disease in guinea pigs which is characterized by lymphadenitis with formation of abscesses in the superficial lymph nodes. The cause of the disease is a hemolytic streptococcus of the *pyogenes* group. The natural infection is chronic and comparatively innocuous, artificial infection is usually acute and rapidly fatal. The disease presents a seasonal variation and occurs more frequently in females than in males. The source and mode of the natural infection are not established. The etiologic significance of the infecting organism has been established.

MALIGNANT TUMORS OF THE ADRENAL IN CHILDREN*

WITH REPORT OF A CASE

BY P A BENDIXEN, M D, AND F H LAMB, M D, DAVENPORT, IOWA

MALIGNANT tumors of the suprarenal glands have been recognized for many years, but it was not until 1907 that Robert Hutchinson¹ "first drew attention to a definite clinical syndrome, occasionally met with in children, consisting of cases of sarcoma of one or the other suprarenal with metastases in bones of the skull"² He then reported ten cases in all, seven of which were published for the first time In view of the unique character of the lesion, the peculiar metastases, the striking clinical symptoms, and their occurrence only in children, it is rather strange that the condition had not received more attention before that time

By way of orientation, it is worth reviewing briefly the relationship between malignant adrenal tumors and the clinical manifestations of these tumors with reference especially to their embryologic origin The adrenal gland is composed of a cortex and medulla, derived from two separate primary germ layers The cortex is of mesoblastic origin while the medulla is of neuroectodermic origin, the latter layer giving rise also to the sympathetic nervous system

Bulloch and Sequeira² in 1905 collected and presented evidence showing the relation of altered suprarenal function and the development of the genital system They discussed twelve cases of malignant neoplasms of the adrenal cortex in children, with additional reports of five necropsies, showing hypergenitalism to be associated with simple hypertrophy of the cortex In another series of cases they pointed out the association of under-developed genitalia with hypoplastic or aplastic cortices,³ confirming the observations of Wiesel⁴ and Karakascheff⁵

Guthrie and Emery⁶ in 1907, called attention to another syndrome in which cortical neoplasms were associated with precocious obesity and muscular hypertrophy

Hutchinson in 1907 reported the series of cases, previously referred to, characterized by medullary tumors of the adrenal with their unique metastases to the bones of the skull Rolleston⁷ compares these selective metastases with the well-recognized association of carcinoma of the thyroid and skeletal metastases According to the observations of Frew⁸, there are "two entirely different clinical syndromes" in this disease with "equally different pathologic features, according to which suprarenal, right or left, had been the site of the primary growth"

Carter⁹ has recently emphasized the generalization that tumors of the suprarenal medulla do not cause sexual prematurity, but usually manifest them-

*Received for publication March 26 1926

selves first by metastases and resulting local symptoms and signs, as illustrated in the report of our case

There are still other groups of adrenal tumors occurring in children, viz, the cases of congenital sarcoma of adrenal and liver in infants, and those cases of primary adrenal tumors not falling into any of the foregoing groups. Extensive references to these may be found in the contribution of Tileston and Wolbach "Tumors of the Adrenal Gland in Children"¹⁰

Since the clinical manifestations of adrenal tumors fall into groups which render possible a diagnosis during life, and since these syndromes in children differ from those of the adult adrenal tumors, the foregoing case is reported as an illustration of one of the most unique groups—that of Hutchinson, with metastases to the skull (and in contrast to that group described by Pepper in which the metastases are entirely in the liver)

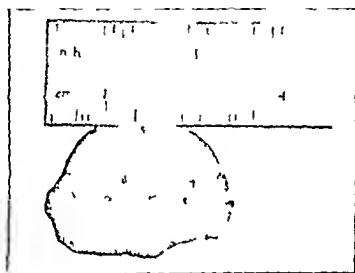


Fig 1—The right adrenal gland gross appearance

REPORT OF CASE

Social Status—An American female child five years of age, entered Mercy Hospital Davenport Iowa, March 12, 1924

Principal Symptoms and Signs—(1) Severe constant pain in the left frontal region of the head (2) Pain in both legs as far as the ankles (3) Loss of weight. (4) Anorexia (5) Exophthalmos of the left eye (6) Ecchymosis of the left lower eyelid

Family History—Father and mother are both well. The mother had a cystic tumor removed from the right ovary and an appendectomy in 1921. The father's mother died of uterine cancer at the age of sixty four years. The patient had three brothers and two sisters all well.

Past Medical History—Patient was a full term baby with normal delivery. She was breast fed for nine months. She had influenza at two months and measles at two years of age. In December, 1923 and again in February, 1924, she fell out of bed, once receiving a slight wound above the left eye, which healed rapidly.

Present Illness—Two and one half months before, the mother noticed a slight protrusion of the left eye with discoloration under the lower lid. The exophthalmos became more and more prominent, and the ecchymosis gradually spread over the entire orbital region. One month after the onset a similar condition began in the right orbit.

The patient moaned during most of her waking hours, cried at times, and complained of severe pain in the head and left side of the face. At times she complained of pain in both thighs and legs. There has been a gradual loss of appetite and emaciation. During the last week of her life she became very apathetic and on account of the bulging forehead the

exophthalmos and ecchymosis, the emaciation, and the apparent hopelessness of the situation, she was truly an object of pity

Physical Examination—Ophthalmoscopic examination by Dr Karl Vollmer, March 12, 1924 media, lens and vitreous clear, no inflammation of the eyeball itself, fundi showed a mild degree of papillitis with some tortuosity of retinal vessels, otherwise fundi were normal, child's vision was very defective On account of the condition and age of the patient, it was difficult to determine the fields or amount of vision She could see objects and persons

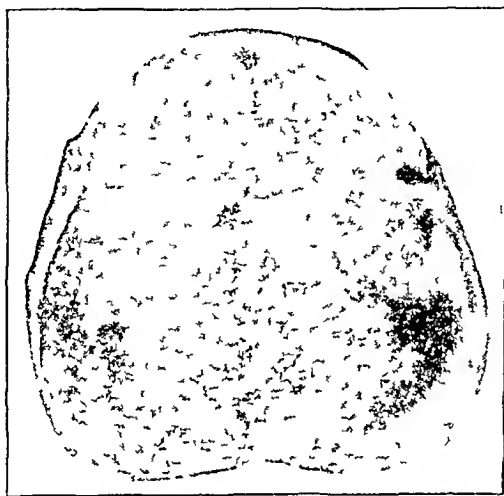


Fig 2—View of inside of skull cap

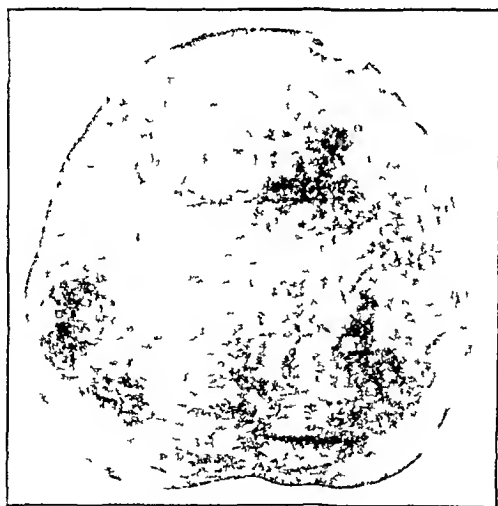


Fig 3—View of outside of skull cap

in the room About one week later, March 17, the exophthalmos increased, the lids did not cover the sclera, the pupils were dilated and reflexes absent The staring, due to the exophthalmos, was straight forward at all times The disc was pale, with slight cupping showing cribriform fascia, a picture of an atrophy following postorbital neuritis

The heart and lung examinations were negative Abdominal findings were negative The presence of a new growth in the abdomen was suspected but could not be found

The extremities were normal with the exception of emaciation The external genitalia were normal

The deep tendon reflexes were present there was no patellar or ankle clonus. The skin reflexes were somewhat diminished.

Laboratory Findings—Blood R B C 2 200 000, W B C 5800 Hgb 35 per cent. Moderate polychromasia and poikilocytosis. No pathologic leucocytes found. Wassermann negative.

Urine—Sp gr 1.020. Chemical and microscopic examinations negative.

Spinal fluid—Pressure 45 mm Hg. Reduction of pressure to 10 mm served to make the child more comfortable but within 24 hours the pressure had returned to 45 mm. There were 4 lymphocytes per cu mm, no globulin, no reduction and a negative Wassermann.



Fig 4.—Lateral view showing fracture of cranial bones.

Clinical Course—The patient entered the hospital March 12 and died April 6 1924. During this time the temperature ranged from 99.6° to 102° axillary. She was very restless and fretful, suffered a great deal of pain and failed rapidly.

AUTOPSY REPORT

Body that of a female child, apparently about six years of age, very emaciated. Skin very pale, dry, and of fine texture. Hair of head is blond, about 12 cm long. There is a prominent bulging of the scalp 8 cm in diameter over the parietofrontal suture in the midline, also similar but smaller bulgings over the course of the parietooccipital sutures on each side and in the midline. The eyes are blue, decidedly protruded, the left slightly more than

the right, the sclerae are dull, pupils irregular, unequal and both dilated. Left upper and lower eyelid slightly everted. There are ecchymotic patches beneath each eye, extending down over the malar eminence, which, in contrast with the very pale skin, and blonde hair gives a grotesque appearance to the facies. The external nasal and auditory orifices, and buccal cavity are negative, mucosa very pale. There are no signs of trauma, wounds, anomalies or deformities.

Removal of scalp by an anterior and posterior flap method reveals dark red, rather firm thickenings of, or infiltrations beneath, the periosteum of the cranium. These are fairly well

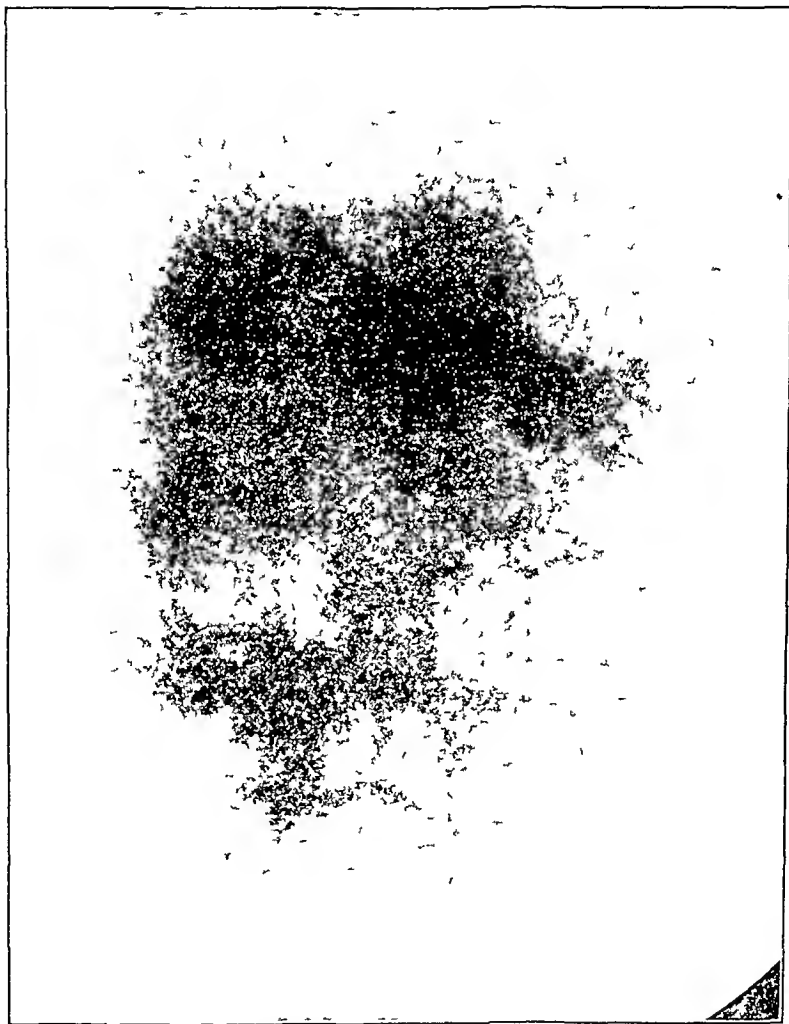


Fig 5—Anterior-posterior of skull showing rarefaction of bones

localized in irregular areas from 4.5 cm to 10.12 cm in diameter, following roughly the courses of the following suture lines—parietofrontal, parietooccipital, and parietotemporal.

Removal of the skull cap shows grossly the same type of infiltrated areas on the inner aspect that were seen on the outer. The dura mater over the hemispheres is somewhat injected, the pia and arachnoid very pale.

The base of the skull shows many large irregular erosions of bone in the anterior and middle cranial fossae about equally extensive on both sides. Upon removing the roof of each orbital cavity it is found that the orbital contents are pushed to one side by a mass of dark

red firm tissue, resembling thickened portions of the skull cap. Chiseling through the floors of both middle cranial fossae reveals this same dark red, firm infiltration extending down through sphenoid sinuses partially filling the maxillary sinuses and anteriorly into the ethmoid sinuses. The foramina in the floor of the middle fossae for the passage of the second, fifth, and sixth cranial nerves are eroded. In fact the bone is almost replaced by infiltrated tissue. It is interesting to note that the dura over the base of the skull is intact the erosive and infiltrative processes being limited to the bone itself, particularly around the foramina and along the suture lines.

The brain is very pale, vessels and venous sinuses containing but very little dark red semiclotting blood. The size of the brain, the convolutions and leptomeninges are normal. The ventricles contain a small amount of lightly turbid fluid and are of normal size. On

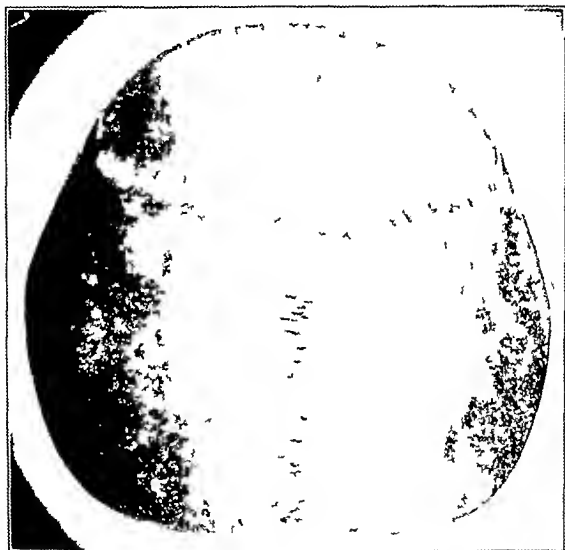


Fig. 6—X ray vault of skull showing rarefaction

section the brain substance is slightly softer than normal and very pale. There are no signs of inflammation, hemorrhage, erosion, or infiltration, the dura having seemed to protect the brain completely from the erosion and infiltration so extensive in the bones of the cranium.

Permission for the autopsy having been limited to the head and later granted for an exploration of the abdomen only, the thorax was not opened.

On median section of the abdomen the subcutaneous fat is scanty, the muscles firm and light red in color. There is no free gas or fluid in the abdominal cavity. The spleen is normal size, soft and pale. The liver normal in size and position is of normal consistency and very pale. The capsule for a patch roughly oval about 5x8 cm just over the right suprarenal glands shows a marked thickening averaging 20 mm. It is white, very firm and resistant. The liver tissue beneath this area shows an area of apparently fatty degeneration 0.5 to 1.0 cm in depth which gradually diminishes to blend with the more nearly normal appearing tissue. The stomach and intestinal canal are negative with the exception of the

very pale color The pancreas seems normal The left kidney is of normal size, capsule stripped easily, consistency normal, outlines between medullary rays and pyramids are well marked, the pelvis and ureter normal The left suprarenal gland is normal in size and apparently so on section The right kidney is similar to the left

A search of the abdomen for evidence of metastases revealed none The retroperitoneal lymph nodes are not enlarged, and are of normal color and consistency Exploration of the large abdominal vessels was negative There are no palpable lymph nodes, either in the abdomen or outside The ribs, bones of the pelvis, and long bones are, so far as can be determined by palpation, negative for signs of metastases

The right adrenal gland is about the size and shape of a large walnut It is not adherent, lies 1 cm above the upper pole of the right kidney, and is in contact with the inferior surface of the liver This area is a white, firm, oval patch in the capsulo as noted above On section, the adrenal is firm, the cut surface is moist and contains a small amount of dark red blood The cortex cannot be distinguished from the medulla The dark red, granular appearance of the cut surface is a decided contrast to the typical yellow, fatty glistening cut surface of the hypernephroma

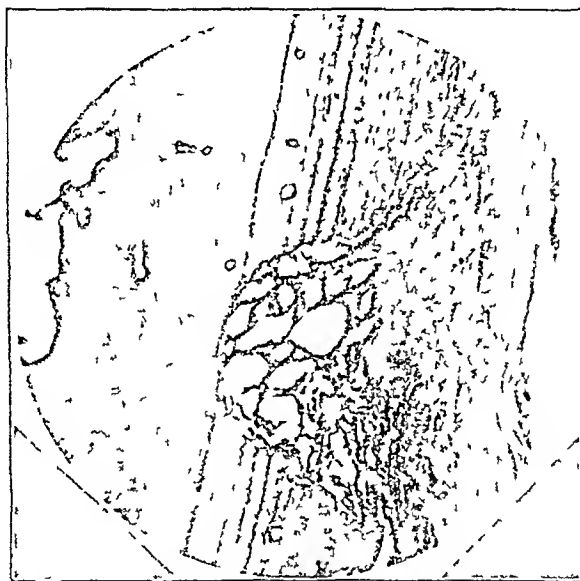


Fig 7—Microphotograph showing invasion of bone

Examination of the tissue removed presented the histology of a typical neuroblastoma The ground work of the tumor was composed of innumerable cells with scarcely perceptible cytoplasm and rounded or somewhat oval, richly chromatic nuclei lying in a homogeneous or finely fibrillated matrix staining pink with eosin Mitotic figures were numerous For the greater part, the cells were distributed diffusely and without any orderly arrangement, although occasionally they tended to form in parallel bundles, especially around the thicker and better formed blood vessels Throughout the tumor at numerous intervals were rosettes made up of a ring of nuclei arranged radiately to a finely fibrillar or structureless substance staining pink with eosin In some instances the rosettes were elongated and gave the appearance of alveoli In some of the rosettes, the nuclei were arranged around structureless, jagged, bluish staining bodies, suggesting calcium deposits The tumor was hemorrhagic, and was frequently traversed by vascular channels, varying in size from that of the most delicate capillary to relatively large sinuses The smaller of these channels were lined by a single layer of endothelium and evidently represented newly formed capillaries, but the larger sinuses were limited externally by compressed tumor cells Free red corpuscles among the tumor cells occurred in great numbers

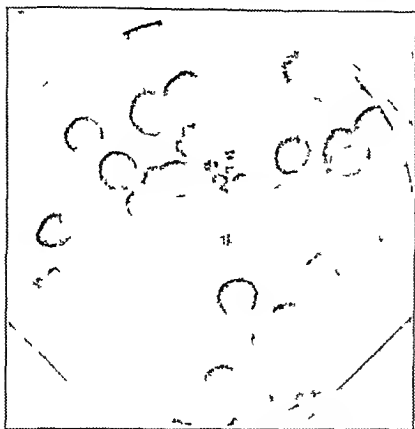


Fig 8—Microphotograph showing mitotic cell figures



Fig 9—Microphotograph—low power of section of right adrenal gland

SUMMARY

The neuroblastoma is a malignant tumor composed of undifferentiated nerve cells or neuroblasts and springs most often from nests of such cells lying in the medulla of the adrenal capsule, but occasionally from identical cells existing in other localities. The tumor presents a characteristic histologic picture marked by the presence of delicate fibrils, supporting cells with scanty cytoplasm and richly chromatic, rounded nuclei; the cells being arranged diffusely or in the form of rosettes around tangled masses of fibrillated or homo-

geneous material, staining pinkish with eosin. In certain cases the fibrils are absent or poorly developed, rosettes cannot be seen, and the very cellular character of the tumor may, in these circumstances, lead to the diagnosis of sarcoma. Based on its cellular unit, the growth in question is most aptly provided for under the designation of neuroblastoma. Knowledge of the true derivation of these growths is of recent date. Virchow first mentioned the possibility. Dalton, Maichand and Kuster gave accurate histologic descriptions, but it was not until 1910 that Wright used the term, "Neuroblastoma," and since then Rich, Wahl, and others have firmly established the origin and histogenesis of these tumors.

CONCLUSION

1 Suprarenal medullary malignancy is not an altogether unusual occurrence.

2 In the majority of cases an orbital hemorrhage is the first sign observed, and it may occur before any tumor is palpable.

3 The orbit first involved is often on the same side as the primary tumor, although this case was an exception.

4 Diagnosis should not be difficult once the orbital hemorrhage has occurred, the disease is likely to be mistaken only for trauma, chloroma and scurvy.

5 Surgical interference can be of no avail, except as a palliative to drain a pyonephrosis or to meet other complications, as the metastases occur usually before a diagnosis can be made.

6 Metastases probably occur via the lymph stream.

7 The malignancy rarely metastasizes to the skin.

8 The medulla of the suprarenal gland being neuroectodermic in origin, these malignant tumors are similar in their histologic structure to malignant neoplasms of the sympathetic nervous system, and they are correctly designated as neuroblastoma.

REFERENCES

- ¹Hutchinson, R. Quart Jour Med, 1907, 1, 33.
- ²Bullock, W., and Sequerra, J. H. Tr Path Soc London, 1905, 111, 189.
- ³Hong, L. A. Am Jour Dis Child, June, 1923, 111, 6.
- ⁴Wiesel. Virchow's Arch f Path Quart, 1904, clxxv, 103.
- ⁵Karakaschew. Ziegler's Beitr z Path Quart, u z Allg Path, 1904, 111, 401.
- ⁶Guthrie and Emery. Tr Clin Soc London, 1907, 11, 175.
- ⁷Rolleston, H. D. Allbutt and Rolleston's System of Medicine, Section on "Ductless Glands," London, 1908, N I, p. 429.
- ⁸Frew, R. S. Quart Jour Med, 1911.
- ⁹Carter, W. E. Am Jour Dis Child, September, 1921, 111, 224.
- ¹⁰Tileston, W., and Wolbach, S. B. Am Jour Med Sc, 1908, clxxv, 871.

A NOTE ON CHLORETONE ANESTHESIA OF DOGS*

By W T DAWSON B A (Oxon) GALVESTON TEXAS

IN 1917 Rowe¹ advised that chloretone should be administered intraperitoneally for dog anesthesia in one of two ways (a) 1 cc per kg of a 40 per cent solution of chloretone in 40 to 45 per cent alcohol (by volume), i.e. 0.4 gm chloretone per kg and (b) after preliminary morphine injection one half this amount of chloretone or 0.2 gm per kg. He advised against intraperitoneal injection in solution in warm oil owing to "slow and uncertain" absorption. We have found that after preliminary morphine injection intraperitoneal administration of 1 cc per kg of a solution of 10 gm of chloretone in 70 cc of ether and 30 cc of liquid petrolatum or 0.1 gm of chloretone per kg quickly produces a satisfactory anesthesia. We have also found that after preliminary morphine injection the intraperitoneal injection of 0.25 gm per kg in warm oil is in a large percentage of dogs followed by death from respiratory failure and that in a number of other cases the anesthesia is very slow in developing.

Chloretone anesthesia was used extensively at the University of Texas during the first semester of 1925 to 1926. Over 100 dogs were prepared for student use in the laboratory of physiology by intraperitoneal injection of chloretone dissolved in warm liquid petrolatum or in cold petrolatum to which ether had been added. Morphine sulphate about 2 to 5 mg per kg was first given subcutaneously. The weight of the dog for this purpose was merely estimated by a glance at the animal. When the dog had become quiet usually in about twenty minutes it was weighed, muzzled if necessary, laid on its back by two students of the group concerned, the hind legs pulled down and injection of chloretone made through the lower half of the ventral abdominal wall sufficiently high up to avoid the bladder. The needles used were always kept sharp. At first 0.25 gm of chloretone per kg was injected, dissolved in warm liquid petrolatum. It was found however that a number of the dogs—sometimes as high as one third—showed respiratory failure before the experiments were well begun. Morphine as is well known depresses the respiratory center, and besides the difficult solubility of chloretone, even when the oil was warmed may have given rise to errors in dosage or to injection of oil at an unreasonably high temperature. The solvent power of the petrolatum was therefore increased by addition of ether. In order to avoid danger of overdose of anesthetic when the ether was added to the solvent, the chloretone dose was reduced. The best results were obtained by the injection of 1 cc of the following anesthetic mixture per kg: 30 cc of liquid petrolatum, 70 cc of ether, 10 gm of chloretone. This corresponds to a dosage of about 0.1 gm of chloretone per kg. After injection the dogs

From the University of Texas School of Medicine Department of Physiology
Received for publication February 24 1926

were left for fifteen minutes before the skin was actually incised, to give ether by inhalation during this period was evidently not advisable because of the large dose given intraperitoneally with the chloretone, the animals were, however, laid out on the board and the sites of operation cleaned and shaved. Additional inhaled ether during operation was sometimes necessary. Early deaths were infrequent.

In addition to the morphine, the animals are evidently under the influence, first, immediately after the injection, of 0.7 c.c. of ether per kg., promptly vaporized (boiling point, 34.6°C) in the peritoneal sac, second, of ether plus chloretone, as the chloretone begins to be absorbed, and finally of chloretone alone as the ether is rapidly excreted through the lungs. The oil has, of course, no anesthetic property and is not required to bring the chloretone into solution, but it appears to make the mixture more easily taken up into the syringe without bubble formation, and also probably in some degree slows the absorption of chloretone after the injection.

Arterial blood pressure was sufficiently high to enable the performance of the usual student laboratory experiments upon factors influencing arterial blood pressure, these included even the effects of hemorrhage and infusion of citrated blood and were repeated several times upon the same animal. Observations upon salivary secretion were found perfectly practicable. The method was found satisfactory also in experiments in which the abdomen was opened, even within fifteen minutes of the intraperitoneal injection, e.g., kidney oncometer experiments, cannulation of the ureters or of the duct of the pancreas or of the bile duct, and preparation of secretin.

The anesthetic solution apparently keeps indefinitely in a well-stoppered bottle. Owing to its ready inflammability it should be kept away from flames during injection.

REFERENCE

- ¹Rowe, L. W. Trichloroethyl Alcohol Anesthesia, *Jour. Pharmacol. and Exper. Therap.*, 1917, ix, 107.

AN INFECTION OF MAN PROBABLY DUE TO SALMONELLA SUIPESTIFER*

By FREDERICK W. SHAW, M.Sc., M.D., RICHMOND, VA

IN 1922 Mackenzie¹ reported a case in which the causative organism described was found to conform in all its reactions to the *Salmonella suispestifer*. Since the available medical literature fails to show another case in which a bacillus of like reactions had been isolated from the blood of man the writer believes the following report will be of interest.

CLINICAL

A man, aged fifty five years a railroad conductor was admitted to Memorial Hospital, Richmond Va. Nov. 18, 1925. He was taken ill on Nov. 15 but continued working until Nov. 17 when he became worse and called a physician. He had a cough and small amount of rusty sputum.

Patient was a well developed well nourished, middle aged white man. He seemed acutely ill and was semicomatose. Respirations fast. Pupils reacted. Heart apparently not enlarged, rate rapid, rhythm regular, sounds clear, no murmur. Lungs percussion note resonant except posteriorly at bases, breathing somewhat stertorous. There were, however, no gross changes, a shower of fine rales (inspiratory) was heard over right base, posteriorly few squeaks scattered throughout. Abdomen soft and distended, no rigidity or apparent tenderness. No edema. Deep reflexes present in both extremities.

Diagnosis—Acute lobar pneumonia (influenzal type). The temperature varied from 104.5° to 105° F., the pulse rate was 125 on admission and increased until it was 140 per minute at 4 A.M. on Nov. 19, respirations were 40 per minute on admission and declined to 25 at 4 A.M. on Nov. 19.

Blood—Wassermann test was negative. There were leucocytes 15,000 (neutrophils 89 per cent, small lymphocytes 8 per cent, large mononuclears 3 per cent). The Widal test was positive in 1 to 20.

The patient died at 11 A.M. on Nov. 19, 1925.

Autopsy—Bilateral chronic adhesive pleuritis, bilateral chronic interstitial pneumonia, multiple acute septic infarcts with beginning abscess formation in the left kidney, cloudy swelling of the liver, spleen and kidneys.

BACTERIOLOGY

The organism (S No. 155) was isolated from the blood stream in pure culture. It was a nonlactose fermenting, nongelatin liquefying, gram negative, motile, nonspore forming rod. Cultures on Russell's double sugar produced acid and gas but with alkaline slant, for this reason they resembled paratyphi and schotmuelleri in the above but it did not agglutinate with the

From the Department of Bacteriology, Medical College of Virginia.
Received for publication February 23, 1926.

antisera for these organisms. This organism was then grown in fermentable substances, the results of which are shown in Table I.

An immune serum, which was prepared with this organism, agglutinated the organism in 1 to 25 600 dilution, but paratyphi and schotmuelleri did not agglutinate in 1 to 40 (Table II).

TABLE II
AGGLUTININS

SERA	ORGANISMS			
	S PARATYPHI	S SCHOTTMUELLERI	S SUIPESTIFER	S #155
S paratyphi	3.00			50-
S schottmuelleri		6400		40-
S suipestifer			51200	51200
S #155	40	40-	25600	25600

TABLE III
AGGLUTININ TESTS

SERA	MIXED WITH	ORGANISM AGGLUTINATED	TITER
S #155	suipestifer	S #155	450-
(original titer 25600)	S #155	suipestifer	900-
S suipestifer	suipestifer	S #155	400-
(original titer 51200)	S #155	suipestifer	400-

Two strains of *Salmonella suipestifer* agglutinated to full titer and absorbed the agglutinins for S No 155.

An immune serum was prepared with one of the suipestifer strains. The homologous organism agglutinated in 1 to 51 200 dilution. S No 155 agglutinated to full titer and absorbed the agglutinins for suipestifer (Table III).

Two rabbits were inoculated intraperitoneally with 0.25 cc of a twenty-four hour broth culture of S No 155. They died on the sixth day. Autopsy showed pale spots on the spleen, mottled liver, small hemorrhages in the lungs, congestion of the vessels in the small intestines and hemorrhagic areas in the peritoneum.

DISCUSSION

It may be seen from Table I that this organism differs from *Salmonella paratyphi* in fermenting, glucose and in not fermenting, arabinose from *S schottmuelleri* and *aertrycke* (Mutton) in not fermenting arabinose and inositol, and from Para "C" *aertrycke* (Newport) and *S enteritidis* in not fermenting arabinose. Also it does not ferment dulcitol until the eighth day, while the six organisms already mentioned ferment dulcitol rapidly. Further, it does not ferment trehalose.

Mackenzie (loc cit) described the cultural characteristics of the organism isolated by him as growing readily on all ordinary laboratory media, nongelatin liquefier and does not produce indol. In litmus milk it produced acid which gave way after forty-eight hours to alkali and the milk remained strongly alkaline. He found that at the end of twenty-four hours' incubation there were acid and gas in the dextrose and maltose fermentation tubes, acid only in mannite while lactose, saccharose and dulcitol remained unchanged.

Staphylococcus albus (hem) 1, *Streptococcus equinus* 1, *Streptococcus mitis* 1, and *Streptococcus subacidus* 1. The streptococci were classified according to Holman's classification, which we have found very satisfactory.

Endocarditis—There were 15 cultures taken in the Medical Wards during the past two years on cases having all the clinical characters of endocarditis. Five or 33.3 per cent were positive. A streptococcus of the viridans type was obtained 3 times, a *Streptococcus mitis* 1, and a *Streptococcus pyogenes* 1.

Meningitis—There were 11 cultures made on meningitis cases and 4 positives resulted from this series or 27.4 per cent. The meningococcus was obtained twice, a streptococcus once and yeast was grown from another case.

Simple Blood Culture—In 28 cases we failed to reveal any organism in the blood stream where the procedure was requested to try to determine the cause of pyrexias of unknown origin.

Reference to Table I will show that no positive results were obtained by culturing the blood of 20 cases clinically diagnosed chronic arthritis.

For some time past we have directed our attention to the method already mentioned which depends upon taking a large quantity of blood and which

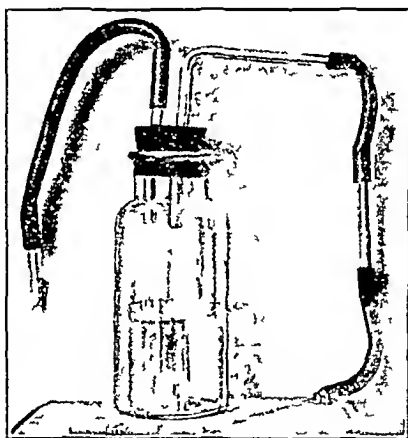


Fig 1—Bottle as prepared for use can be assembled cheaply in any laboratory

we speak of as the *massive method* to emphasize the amount to be withdrawn from the vein. Fifty cubic centimeters have been the minimum, but many times 100 cc or more have been taken. This blood is collected into a 200 cc wide mouth bottle equipped with a tightly fitting rubber stopper with 2 perforations. Through each hole passes a glass connection, one going to the needle, the other to a rubber tubing fitted with a glass mouthpiece through which suction is made by the operator to facilitate the flow of blood from the vein. The blood is collected in the usual way in the bottle containing 100 cc of sterile doubly distilled water and 3 gm of sodium citrate. On referring to the literature we find that Lintz in 1913 described a similar but somewhat less simple apparatus.

After the blood is drawn into the bottle, which is concealed from the patient's view under a sterile cover, it is taken directly to the laboratory for inoculation into media. This avoids a display of test tubes, media, etc,

at the patient's bedside. Indeed, so simple is the collection and transportation of blood by this method that it lends itself to the use of the general practitioner with whom blood cultures have, on the whole, failed to become popular. In the laboratory the blood is placed in large tubes and centrifuged for twenty minutes at high speed. Under the greatest precautions the sediment is used for seeding into media. The blood will be defibrinated and almost completely laked by the citrate water. The broken cells will act as a mechanical filter and drag to the bottom the bacteria in the blood. Transfer of sediment to culture media may be done in one of two ways: the supernatant liquid may be poured away leaving at the bottom a fairly solid layer of reddish gray material or a sterile pipette may be plunged through the upper layers and the sediment collected in it. A long tubing with a glass



Fig. 1.—Bottle wrapped as still life for use

mouthpiece, attached to the pipette, facilitates the removal of the sediment. Deep serum water broth, plain and blood agar plates constitute the ordinary media used. In cases where we suspected the typhoid colon group, bile enrichment media have been used. Glucose broth has also been used to advantage. Brain medium has been used at times but with no conspicuous advantage.

We have had an opportunity to take 34 blood cultures by this so called *massive method*. We report 14 positive results or 41 per cent and 20 negative or 59 per cent. The cases upon which this method was used, those with previous negative results or with little promise of positive outcome by the ordinary technique, consisted of subacute endocarditis, pelvic inflammation, pyelitis, tonsillitis, acute respiratory infections, chronic arthritis, Hodgkin's disease and neoplasms.

Staphylococcus albus (hem) 1, *Streptococcus equinus* 1, *Streptococcus mitis* 1, and *Streptococcus subacidus* 1. The streptococci were classified according to Holman's classification, which we have found very satisfactory.

Endocarditis—There were 15 cultures taken in the Medical Wards during the past two years on cases having all the clinical characters of endocarditis. Five or 33.3 per cent were positive. A streptococcus of the viridans type was obtained 3 times, a *Streptococcus mitis* 1, and a *Streptococcus pyogenes* 1.

Meningitis—There were 11 cultures made on meningitis cases and 4 positives resulted from this series or 27.4 per cent. The meningococcus was obtained twice, a streptococcus once and yeast was grown from another case.

Simple Blood Culture—In 28 cases we failed to reveal any organism in the blood stream where the procedure was requested to try to determine the cause of pyrexias of unknown origin.

Reference to Table I will show that no positive results were obtained by culturing the blood of 20 cases clinically diagnosed chronic arthritis.

For some time past we have directed our attention to the method already mentioned which depends upon taking a large quantity of blood and which

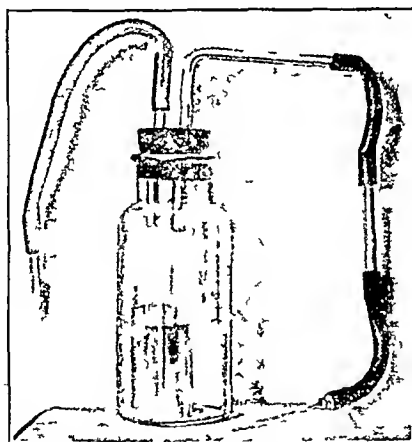


Fig 1—Bottle as prepared for use can be assembled cheaply in any laboratory.

we speak of as the *massive method* to emphasize the amount to be withdrawn from the vein. Fifty cubic centimeters have been the minimum, but many times 100 c.c. or more have been taken. This blood is collected into a 200 c.c. wide mouth bottle equipped with a tightly fitting rubber stopper with 2 perforations. Through each hole passes a glass connection, one going to the needle, the other to a rubber tubing fitted with a glass mouthpiece through which suction is made by the operator to facilitate the flow of blood from the vein. The blood is collected in the usual way in the bottle containing 100 c.c. of sterile doubly distilled water and 3 gm. of sodium citrate. On referring to the literature we find that Lantz in 1913 described a similar but somewhat less simple apparatus.

After the blood is drawn into the bottle, which is concealed from the patient's view under a sterile cover, it is taken directly to the laboratory for inoculation into media. This avoids a display of test tubes, media, etc.,

antisera for these organisms. This organism was then grown in fermentable substances, the results of which are shown in Table I.

An immune serum, which was prepared with this organism, agglutinated the organism in 1 to 25,600 dilution, but paratyphi and schottmuelleri did not agglutinate in 1 to 40 (Table II).

TABLE II
AGGLUTININS

SERUM	ORGANISMS			
	S. PARATYPHI	S. SCHOTTMUELLERI	S. SUIPESTIFER	S. #155
S. paratyphi	3200			40-
S. schottmuelleri		6400		40-
S. supestifer			51200	51200
S. #155	40-	40-	25600	25600

TABLE III
AGGLUTINATION TITERS

SERUM	ABSORBED WITH supe titer	ORGANISM AGGLUTINATED	TITER
S. #155 (original titer 25600)	S. #155 supe titer	S. #155 supe titer	400-
S. supestifer (original titer 51200)	S. supe titer S. #155	S. #155 supe titer	400-

Two strains of *Salmonella* *suipestifer* agglutinated to full titer and absorbed the agglutinins for S. No. 155.

An immune serum was prepared with one of the *suipestifer* strains. The homologous organism agglutinated in 1 to 51,200 dilution. S. No. 155 agglutinated to full titer and absorbed the agglutinins for *suipestifer* (Table III).

Two rabbits were inoculated intraperitoneally with 0.25 cc of a twenty-four hour broth culture of S. No. 155. They died on the sixth day. Autopsy showed pale spots on the spleen, mottled liver, small hemorrhages in the lungs, congestion of the vessels in the small intestines and hemorrhagic areas in the peritoneum.

DISCUSSION

It may be seen from Table I that this organism differs from *Salmonella* *paratyphi* in fermenting xylose and in not fermenting arabinose, from *S. schottmuelleri* and *aertryele* (Mutton) in not fermenting arabinose and inosite, and from *Para 'C' aertryele* (Newport) and *S. enteritidis* in not fermenting arabinose. Also, it does not ferment dulcitate until the eighth day, while the six organisms already mentioned ferment dulcitate rapidly. Further, it does not ferment trehalose.

Maletzkie (loc. cit.) described the cultural characteristics of the organism isolated by him as growing readily on all ordinary laboratory media, nongelatin liquefier, and does not produce indol. In litmus milk it produced acid which gave way after forty-eight hours to alkali and the milk remained strongly alkaline. He found that at the end of twenty-four hours' incubation there were acid and gas in the dextrose and maltose fermentation tubes, acid only in mannite, while lactose, saccharose and dulcitate remained unchanged.

TABLE I

	MOTILITY	GELATIN	CIV	MILK	LACTOSE	SACCHAROSE	DULCIFY	MANNITE	DELTATOSE	MALTATOSE	MALTATOSE	DEVTATRY	RAFFINATOSE	ARABINATOSE	TRIPHATATOSE	INULIN	SOPRIT	GALACTATOSE	LEVULOSE	INOSITE	SALICIN	ADIVGD VIN	GLYCERIN	RHAMATOSE	XYLOSE	LEAD ACETATE	VOGES PROS	INDOL
S paratyphi	+	-	-	A	O	O	AG	AG	AG	AG	AG	O	O	AG	AG	O	O	AG	AG	O	O	O	O	AG	O	O	O	O
S schottmulleri	+	-	-	A	O	O	AG	AG	AG	AG	AG	O	O	AG	AG	O	O	AG	AG	AG	AG	O	O	AG	B	O	O	O
Parityphoid "C"	+	-	-	A	O	O	AG	AG	AG	AG	AG	O	O	AG	AG	O	O	AG	AG	O	O	O	O	AG	AG	B	O	O
S enteritidis	+	-	-	A	O	O	AG	AG	AG	AG	AG	O	O	AG	AG	O	O	AG	AG	O	O	O	O	AG	AG	B	O	O
S aertrycke (New port)	+	-	-	A	O	O	AG	AG	AG	AG	AG	O	O	AG	AG	O	O	AG	AG	O	O	O	O	AG	AG	O	O	O
S aertrycke (Mutton)	+	-	-	A	O	O	AG	AG	AG	AG	AG	O	O	AG	AG	O	O	AG	AG	AG	AG	O	O	AG	AG	O	O	O
S supestifer	+	-	-	A	O	O	O ¹	AG	AG	AG	AG	O	O	O	O	O	O	AG	AG	AG	O	O	O	AG	AG	O	O	O
Mackenzio	+	-	-	A	O	O	O ²	AG	AG	AG	AG	O	O	O	O	O	O	AG	AG	AG	O	O	O	AG	AG	O	O	O
#175 M C V	+	-	-	A	O	O	O ²	AG	AG	AG	AG	O	O	O	O	O	O	AG	AG	AG	O	O	O	AG	AG	O	O	O

¹Some strains produce acid and gas
²Acid and gas on eighth day
B = Blackened A = Acid G = Gas A, Al = Acid then alkaline

OBSERVATION ON BLOOD CULTURES WITH A SPECIAL REFERENCE TO THE QUANTITY OF THE BLOOD USED*

By HERBERT FOX M D AND WILLIAM G LEAMAN M D, PHILADELPHIA, PA

THE following analyses have recently been made with the idea of discovering the usefulness of our blood culture technique and since some of the observations may be helpful to others it has seemed well to put our material on record. Comparison of data from one source of information with those from another may or may not be possible since percentage results of blood culture success and failure depend upon many things. One of these factors as we shall try to point out is the quantity of blood used. Other factors of great importance are the types of cases tested the activity or stage of the process the technique and the media used and the experience of the operator. Strict comparison can only be made when all factors are known and evaluated. Repeated cultures on the same case will increase the percentage of positives for a disease but may be misleading for the total of blood cultures taken. It is obvious that the oftener an attempt is made the greater is the probability of discovering circulating bacteria. So too we think, it will be admitted that the greater the amount of blood taken the greater the chance of growing bacteria therefrom. Our data show an increase of findings when this has been done. Blood cultures taken by the usual technique are analyzed for the purpose of recording routine results. When, however, the examination has been negative in the presence of evidence suggesting a bacteremia a large quantity of blood has been withdrawn, the amount safe for the patient being decided in conference between the clinician and the laboratory man. Repeated cultures may be necessary even by this method, but a negative result certainly has a greater significance.

A series of 321 cultures was made by the following simple method. From 10 to 15 c.c. of blood were withdrawn and blood agar plates and broth bottles inoculated in the usual way at the bedside. Of the 321 cultures made in this way 65 or 25.4 per cent were positive and 256 or 74.6 per cent negative.

Typhoid—There were 27 cultures made from the blood of patients diagnosed typhoid fever clinically and who showed a positive Widal reaction at one stage or other of the disease. Of the 27 cultures 12 or 44.5 per cent were positive and 15 or 55.5 per cent were negative. They were all grown in bile enrichment media.

Pneumonia—There were 58 cultures of the series made on pneumonia cases of various types on the medical side of the hospital and from 16 or 27 per cent of these cases the pneumococcus was recovered. Five were Type I four were Type II one Type III and six Type IV.

Puerperal Sepsis—There were 50 cultures of the series made in the Maternity Wing on cases diagnosed sepsis. Of these 17 or 30.3 per cent were positive and 39 or 69.6 per cent negative. *Streptococcus pyogenes* appeared 7 times, *Staphylococcus aureus* (hem.) 6 times.

From the William Pepper Laboratory of Clinical Medicine University of Pennsylvania.
Received for publication February 27 1916

Staphylococcus albus (hem) 1, *Streptococcus equinus* 1, *Streptococcus mitis* 1, and *Streptococcus subacidus* 1. The streptococci were classified according to Holman's classification, which we have found very satisfactory.

Endocarditis—There were 15 cultures taken in the Medical Wards during the past two years on cases having all the clinical characters of endocarditis. Five or 33.3 per cent were positive. A streptococcus of the viridans type was obtained 3 times, a *Streptococcus mitis* 1, and a *Streptococcus pyogenes* 1.

Meningitis—There were 11 cultures made on meningitis cases and 4 positives resulted from this series or 27.4 per cent. The meningococcus was obtained twice, a streptococcus once and yeast was grown from another case.

Simple Blood Culture—In 28 cases we failed to reveal any organism in the blood stream where the procedure was requested to try to determine the cause of pyrexias of unknown origin.

Reference to Table I will show that no positive results were obtained by culturing the blood of 20 cases clinically diagnosed chronic arthritis.

For some time past we have directed our attention to the method already mentioned which depends upon taking a large quantity of blood and which

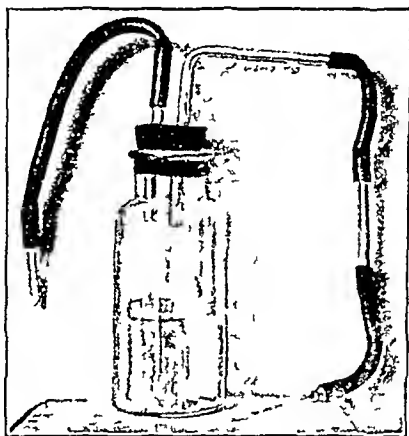


Fig 1—Bottle as prepared for use can be assembled cheaply in any laboratory.

we speak of as the *massive method* to emphasize the amount to be withdrawn from the vein. Fifty cubic centimeters have been the minimum, but many times 100 cc or more have been taken. This blood is collected into a 200 cc wide mouth bottle equipped with a tightly fitting rubber stopper with 2 perforations. Through each hole passes a glass connection, one going to the needle, the other to a rubber tubing fitted with a glass mouthpiece through which suction is made by the operator to facilitate the flow of blood from the vein. The blood is collected in the usual way in the bottle containing 100 cc of sterile doubly distilled water and 3 gm of sodium citrate. On referring to the literature we find that Lutz in 1913 described a similar but somewhat less simple apparatus.

After the blood is drawn into the bottle, which is concealed from the patient's view under a sterile cover, it is taken directly to the laboratory for inoculation into media. This avoids a display of test tubes, media, etc.,

at the patient's bedside. Indeed, so simple is the collection and transportation of blood by this method that it lends itself to the use of the general practitioner with whom blood cultures have, on the whole, failed to become popular. In the laboratory the blood is placed in large tubes and centrifuged for twenty minutes at high speed. Under the greatest precautions the sediment is used for seeding into media. The blood will be defibrinated and almost completely lysed by the citrate water. The broken cells will act as a mechanical filter and drag to the bottom the bacteria in the blood. Transfer of sediment to culture media may be done in one of two ways: the supernatant liquid may be poured away leaving at the bottom a fairly solid layer of reddish gray material or a sterile pipette may be plunged through the upper layers and the sediment collected in it. A long tubing with a glass

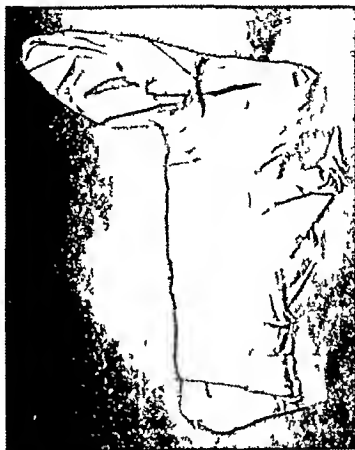


Fig. 2.—Bottle wrapped as illustrated for use.

mouthpiece, attached to the pipette facilitates the removal of the sediment. Deep serum water broth, plain and blood agar plates constitute the ordinary media used. In cases where we suspected the typhoid colon group, bile enrichment media have been used. Glucose broth has also been used to advantage. Bram medium has been used at times but with no conspicuous advantage.

We have had an opportunity to take 34 blood cultures by this so-called *massive method*. We report 14 positive results or 41 per cent and 20 negative or 59 per cent. The cases upon which this method was used, those with previous negative results or with little promise of positive outcome by the ordinary technique, consisted of subacute endocarditis, pelvic inflammation, pyelitis, tonsillitis, acute respiratory infections, chronic arthritis, Hodgkin's disease and neoplasms.

TABLE I
SUMMARY OF 321 BLOOD CULTURES BY THE SIMPLE METHOD

DIAGNOSIS	POSITIVE	NEGATIVE	PER CENT POSITIVE
Typhoid	12	15	44.5
Meningitis	4	7	36.3
Pyonephroses	1	1	50
Puerperal Sepsis	17	39	30.3
Endocarditis	5	10	33.3
Pneumonia	16	42	27.0
Septicemia	4	12	25.0
Phlebitis	1	2	33.0
Rheumatic Fever	1	4	20.0
Mastoiditis	3	7	30.0
Cellulitis	1	7	12.5
Parotitis	0	2	00.0
Erysipelas	0	3	00.0
Pyrexias Unknown Origin	0	28	00.0
Purpura	0	12	00.0
Perniciou Anemia	0	10	00.0
Chronic Arthritis	0	20	00.0
Empyema	0	2	00.0
Post Operative Wound Infection	0	18	00.0
Pyelitis	0	15	00.0
	65	256	

TABLE II
MASSIVE BLOOD CULTURES

DIAGNOSIS	POSITIVE	NEGATIVE	PER CENT POSITIVE
Subacute Endocarditis	4	1	80
Chronic Arthritis	2	4	33.3
Hodgkin's Disease	1	2	33.3
Lymphosarcoma	1	1	50
Pyelitis	3	1	75
Respiratory Infection	1	2	33
Rheumatic Fever	0	2	0
Mononucleosis	0	2	0
Pelvic Inflammation	1	1	50
Tonsillitis	0	2	0
Chronic Osteomyelitis	1	2	33
	14	20	

This technic gives, therefore, about 16 per cent better results than does the simple routine method. Its value is especially definite in cases of endocarditis where we obtained 80 per cent of positive cultures. This figure can almost certainly be improved upon.

A diphtheroid was obtained from a patient suffering from Hodgkin's disease and from a case of lymphosarcoma. Cases of acute pyelitis yielded positive cultures in three out of four instances or a percentage of 75. Colon bacillus was found once, *B. mucosus capsulatus* once and a hemolytic *Staphylococcus* once. The discovery of a number of the *B. capsulatus* group is most unusual and worthy of record. Rhea and Emmons also report its finding. It is not, however, characteristic for this organism to invade the blood stream, although it doubtless plays an important rôle in carrying on inflammation of the pelvis and of the bladder whence it might occasionally enter the circulation. The colon bacillus was also obtained once from a case of pelvic suppuration, a subsequent culture three weeks later was done on this case and was negative.

The patient was clinically improved, and we had no reason to doubt our original finding

Perhaps the most interesting of our findings occurred in an acute respiratory tract infection, resembling influenza complicated by tonsillitis. From this blood by the *massic technique* there grew *Streptococcus mitis*, *Micrococcus catarrhalis*, and a diptheroid. A repetition of the culture four days later resulted in no bacterial growth whatever. The streptococcus was identified by the Holman scale, the *M. catarrhalis* by the Elser Hinton criteria. The finding of this coccus in the blood is most unusual and though undoubted is held *sub judice* until more data are available. Canon (*Bakteriologie des Blutes*, 1905) credits the possibility of the entrance of this gram negative group from the pharynx into the blood.

A case of chronic osteomyelitis with discharging sinuses yielded a hemolytic *Staphylococcus aureus* whereas two similar cases were negative. Results were negative in two cases of acute tonsillitis with adenitis.

We do not wish to draw conclusions from this short series of blood cultures but we venture to suggest that greater satisfaction will be experienced by both clinicians and laboratory workers if larger quantities of blood are used, especially if the bacterial contents be concentrated by hemolysis, defibrination and centrifuging. The method and the bottle that we describe are less cumbersome than others given in the literature and are practicable for clinicians in private practice if they will take a very little extra trouble. Certainly it seems from our experience that a higher percentage of findings will result and that a greater variety of bacteria will be discovered by the use of a large quantity of blood. Such a technique may obviate a repetition of blood culture. The growth of bacteria is no more rapid by this method, but, of course, when a larger number are present in the original seeding the mass of growth is greater and more quickly perceptible. By this technique negative cultures seem to have more significance.

REFERENCES

- Edsall Clinical Value of Blood Cultures *Pennsylvania Med Jour* 1905 1906 ix 844
 Fowler A Short Note on Blood Cultures *Jour Roy Army Med Corps Lond* 1912, xviii 574
 Gerstley, Jesse R. and Fuller Harry B. A Simple Technique for Blood Cultures *Jour Am Med Assn* 1914 lxi 376
 Heffernan and Clarke Blood Culture and Clinical Diagnosis *Jour Royal Army Med Corps Lond* 1915 xxv 229
 Hubst The Value of Blood Culture in the Diagnosis of Infectious Disease *Long Island Med Jour*, 1913 vii 7
 Judd and Simon The Vacuum Tube of Kiedel as Applied to Blood Culture Work, *Jour Am Med Assn*, 1915 lxi 822
 Libman General Infections by Bacteria *Jour Michigan Med Soc*, November, 1924, xxiii, 462
 Lantz Blood Cultures Simplified by a New Apparatus *Long Island Med Jour*, 1913, vii, 60
 Macleod Bevan and Brown The Technique of Blood Culture, *Jour Path and Bacteriol* 1918 xxii 74
 Mann, Rainsford, and Warren Report of Blood Cultures Examined in the Pathological Laboratory of the Roosevelt Hospital, *Med. and Surg. Rep. Roosevelt Hosp.*, 1915 231
 McJunkin A Tube for Collecting Blood for Cultures *Jour Am Med Assn* 1914 lxi 774
 Rhea Lawrence J. and Emmons Arthur B. A Series of 91 Consecutive Blood Cultures *Boston Med and Surg Jour* 1907 clvi 809
 Rythenberg Charles Value of the Use of Ammonium Oxalate in Blood Culture-Technic *Proc N Y Path Soc* 1908 1909, N S, viii 192

- Rythenberg, Charles Jour Med Research, 1909, p 79
 Straus, Aubrey H Blood Cultures as a Diagnostic Aid, Virginia Med Month, January, 1924, 1, 701
 Straus Significance of Negative Blood Culture Depends on Method Used, JOUR LAB AND CLIN MED, February, 1924, ix, 345
 Straus and Bird L C Practical Use of Blood Culture in Public Health Work Am Jour Public Health, May, 1924, xiv, 413
 Thornley Notes on Blood Culture Technique, Brit Med Jour, 1916, xi, 555
 Weston, Paul G A Simple Method for Blood Culture, Jour Am Med Assn, 1916, lxxvi, 507

NOTE ON THE QUANTITATIVE DETERMINATION OF ARSENIC IN ORGANIC MATERIAL*

By ALLAN WINTER ROWE, PH D, BOSTON, MASS

A PROCEDURE frequently requested in the conduct of the general hospital laboratory is the determination of arsenic in body fluids or in other organic material. The amounts present are seldom large, and in certain parts of the country, at least, traces of arsenic are to be regarded as a normal constituent of the substances in question. It is possible in a district such as New England that the widespread use of arsenate of lead in combating the ravages of the gypsy moth is responsible for the condition. Equally it may be that minute quantities of arsenic are intrinsically a normal constituent of the human body as claimed by Gautier. In any case, with arsenic possibly present as a normal constituent, only quantitative methods are of value and these to ascertain if the amount of the offending material observed be outside what may be designated as normal limits. The present method here presented is an adaptation of the method first defined by Sanger and Black¹ and later modified by W. A. Boughton². It is presented here in this form primarily for the benefit of those who desire a sensitive, accurate and ready means of quantitating arsenic in the presence of much organic material.

This method consists primarily of two independent procedures, one being the extraction of the arsenical material and the other the actual estimation of the arsenic.

EXTRACTION

The entire amount of urine or body fluid to be examined is concentrated on a steam bath, to a volume of 50 to 75 cc., and a sufficient amount of arsenic-free hydrochloric acid is added to form the constant boiling mixture of the acid solution (20 per cent HCl solution).

The practice in this laboratory is to carry out the evaporation in a porcelain or Pyrex evaporating dish and to wash the concentrated material into the distilling flask of the apparatus shown in Fig 1, first with a small amount of water and then with the hydrochloric acid.

The solution is now boiled, and the distillate is collected under an amount of ammonium hydroxide slightly larger than the theoretical amount required

*Communication from the Evans Memorial Boston Massachusetts
 Received for publication March 2 1926

to neutralize the hydrochloric acid distilled over (about 100 cc of 28 per cent NH_4OH). At the conclusion of the operation the stopcock is opened to release the negative pressure and prevent sucking back of the distillate.

This method with a slight modification can be used for the extraction of arsenic from such solid organic material as finely chopped muscle hair, felt, and similar substances. The process is modified merely in that the arsenic free hydrochloric acid of the highest concentration (about 37 per cent) is added directly to the organic material in the distillation flask without dilution in order to liberate the arsenic content. In using the strong

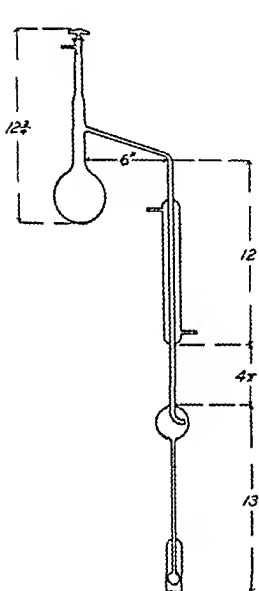


Fig. 1

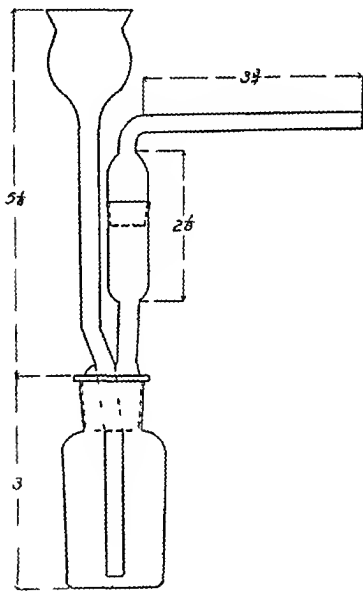
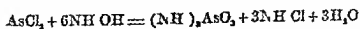


Fig. 2

acid it may be necessary to attach an ammonium chloride absorption tube to the ammonium hydroxide receiving apparatus in order to avoid the possibility of mechanical loss of arsenic trichloride. The absorption tube is not needed when distilling a constant boiling acid mixture as very little gaseous hydrochloride is evolved.

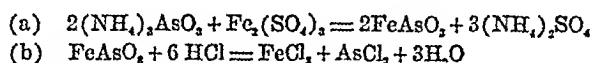
The arsenic is distilled over as the chloride and presumably forms ammonium arsenite as expressed by the equation



The arsenic is now precipitated from solution by the addition of 2 to 3 cc of saturated $\text{Fe}(\text{SO}_4)_3$ and filtered. The precipitate is dissolved in the

cold in as small amount of 10 per cent HCl as possible and transferred to the hydrogen generator

The operations thus involved may be represented diagrammatically by the equations



Another and equally tenable hypothesis assumes that ferric arsenite is too soluble to allow the precipitation and collection of the very small amounts of arsenic with which this method deals and that instead of the above reactions the arsenic may be adsorbed on ferric hydroxide formed when ferric sulphate is added to the ammoniacal solution

It has been found that magnesium hydroxide is almost as efficient as ferric hydroxide in the collection of arsenic. These observations are in perfect harmony with current therapeutic practice

QUANTITATION

Three grams of uniformly granulated zinc are placed in the bottle of the hydrogen generator (Fig 2) and a strip of sensitized paper (v1) in the 41 mm deposition tube. A plug of absorbent cotton that has been kept over concentrated sulphuric acid and a loose plug of cotton that has been moistened with lead acetate and dried, are placed in the enlargements of the exit tubes. An hour's preliminary run is necessary to moisten the cotton partially. Add 15 c c of diluted hydrochloric acid (1-6), and let the hydrogen pass for ten to twenty minutes to make sure the reagents cause no stain. Add the whole or an aliquot part of the solution to be tested. Arsenic will produce a color on the paper in a few minutes, and this will reach a maximum within thirty minutes. The color of the arsenic bands may be developed either (1) by placing the paper in hydrochloric acid (1:1) for two minutes at a temperature of not over 60° C, or (2) by treating for a few minutes with concentrated ammonium hydroxide. The amount of arsenic is determined by comparison with standard bands prepared by treating test paper by operating the apparatus, having added definite amounts of arsenious oxide, and developing the color as above.¹

The sensitized paper is prepared by soaking strips 4 mm wide, of a hot pressed-paper made by Whatman, in a 5 per cent solution of recrystallized mercuric chloride. These are dried, cut into strips 7 cm long, and protected from light and moisture in a stoppered bottle containing granular calcium chloride, covered with cotton.

It is needless to say that throughout these several operations not only must every care be exercised to avoid contamination of material but that all of the several reagents must be carefully tested to exclude the presence of arsenic in each of them. The method as here indicated is rapid, highly sensitive, and apparently accurate to a high degree, as shown by the recovery of known amounts of arsenic.

REFERENCES

- Sanger and Black Proc Am Acad Arts and Sc, 1907, xlii, 297
 Boughton Personal communication, The method described here is essentially that developed by Mr Boughton We take great pleasure in acknowledging our basic indebtedness to his generous suggestion and cooperation

THE INFLUENCE OF MAGNESIUM SALTS ON AMBOCEPTOR AND COMPLEMENT TITRATIONS*

BY WILFRED H KELLOGG M D AND L AMY WELLS A B, BERKELEY, CALIF

EVERY serologist can recall occasions of failure in satisfactory clearing of tubes that should show complete hemolysis in the Wassermann test and also of trouble with unsatisfactory titrations of complement and amboceptor. The unexpected and often unexplainable fall in the usual titer of hemolysin has been particularly troublesome in systems employing a light cell suspension as in the Kolmer method.

We have uniformly traced our difficulties to salt solution and have solved them temporarily by trying different brands of salt and sometimes different lots of the same brand, we have often noted that a chemically pure product may not give as good results as a commercial product. Mason and Sanford¹ consider that the P_H of the saline is the important factor. This seemed very plausible, and for a time we regarded this explanation as adequate, but as time went on, we were unable to correct our difficulties from this angle. That the P_H alone is not much of a factor is suggested by some of the experimental titrations in the article already referred to. In their Table I a salt solution made from tap water not boiled, with a P_H of 7.8, gave an amboceptor titration of 16000, while a solution made with double distilled water (Table II) and having a P_H exactly the same gave a titration recorded as nil. The result here was evidently due to some difference between tap and distilled water, other than the mere matter of hydrogen ion concentration. This difference we have found to depend on the presence of magnesium, which is in most natural waters. That a fairly wide range in the P_H of the salt solution is consistent with the occurrence of usual results is shown by the 13000 amboceptor titration obtained by the authors quoted (Table III) in an artificially acidified tap water solution having a P_H of 5.8. Incidentally, this experiment also shows the influence of magnesium in the tap water, for the titration of 3000 undoubtedly would not have been reached in such an acid medium without it.

We have performed many experiments with various lots of sodium chloride and have included variations in the P_H values as well as slight variations in the tonicity of the solutions and tap, distilled, and double distilled waters were used. There is nothing to be gained in tabulating these experiments since nothing definite resulted beyond additional confirmation of the idea that reaction in itself is relatively unimportant. One particular lot of salt gave

*From the State Hygienic Laboratory University of California Berkeley California.
 Received for publication March 13 1926

such uniformly high titrations that it was decided to search for a chemical difference between this and lots of sodium chloride. The outstanding characteristic of this brand of salt was found to be the presence of considerable amounts of magnesium. The next step was to analyze the tap water, which also gave high titrations with chemically pure sodium chloride, and it was found to contain 14 parts per million of magnesium. This quantity, estimated as chloride, represented 0.12 gm per liter. Experiments with physiologic saline solution made with chemically pure salt, distilled water, and varying amounts of magnesium chloride showed that less than 0.02 gm per liter were without effect or had little effect on the titration but that 0.05 and over markedly raised the titer of amboceptor over that obtained with salt solution not containing magnesium. The upper limit for the addition of magnesium chloride was reached when a mixture of isotonic sodium chloride solution and a mixture of isotonic magnesium chloride (19.0 gm of moist crystals per liter) in the proportion of 98 parts of the former and 2 parts of the latter, was used. Any further addition of magnesium beyond this point resulted in a rapid fall in the titer of amboceptor until absolute inhibition of hemolysis

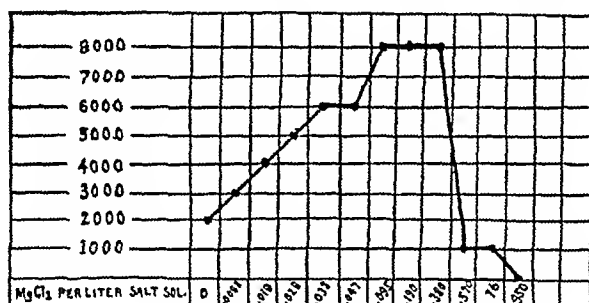


Fig 1 — Amboceptor titrations

was reached with 5 parts of the magnesium solution to 95 parts of normal salt solution. The influence of varying the content of magnesium chloride from minimum to maximum is graphically shown in the profile line chart (Fig 1) in which the amount of magnesium present is shown in grams per liter.

The question naturally arose as to whether the favorable action of magnesium was due to the base itself, to the base in combination with certain acid radicals, or whether other bases might not also be effective. Accordingly various other salts were tried then selection being influenced by such obvious associations or resemblances as chemical grouping with other metals, atomic weights, etc. Table I shows the results of titrations conducted with various magnesium salts, from which it appears that the base is the essential factor.

TABLE I
AMBOCEPTER TITRATION

	WATER	SALT	ADDED CHEMICAL*	AMBOCEPTER TITRATION
1	Distilled	Merck's CP	Magnesium Chloride 08	1 8000
2	Distilled	Merck's CP	Magnesium Sulphate 08	1 8000
3	Distilled	Merck's CP	Magnesium Citrate 10	1 8000
Control	Distilled	Merck's CP	none	1 3000

*Per 1000 c c

Manwaring noted the inhibiting action of certain salts, including $MgCl$, and found that no change of the corpuscles or amboceptor occurred and that by precipitating out the inhibiting salts, the hemolytic power of the mixture was restored, this showed that the complement was not destroyed. He considered that the action was due to the formation of simple ion complement compounds that are hemolytically inactive. Similar conclusions are reached by Helten and Ruediger. These observations may have a bearing on the question in strengthening our opinion that the action of magnesium in favoring hemolysis is not due to any chemical or physical action on the red cells.

TABLE II

	WATER	SALT	ADDED CHEMICAL OR LITER	PER	AMBOCEPTOR TITRATION
1	Distilled	Merck's CP	Calcium Chloride	08	1 000
2	"	"	Calcium Chloride	20	1 3000
3	"	"	Ammonium Chloride	08	1 3000
4	"	Merck's USP	Zinc Chloride	08	nil
5	"	"	Zinc sulphate	05	nil
6	"	Merck's CP	Strontium Chloride	08	1 1000
7	"	"	Copper Sulphate	08	nil
8	"	"	Barium Nitrate	08	1 2000
9	"	"	Manganese Chloride	08	nil
10	"	"	Magnesium Chloride	08	1 8000
11	Tap	"	None		1 8000

Table II shows the results of experiments with other salts. The explanation of the behavior of magnesium salts in the specific hemolysis of red blood cells is a subject for speculation and further investigation. We have at present no explanation to offer. The idea of catalysis suggests itself, but in this type of reaction it would be an unfamiliar manifestation of such an influence. Some chemical weakening of the erythrocytes with increased vulnerability to lytic agencies is also to be thought of. Magnesium being one of the alkaline earth metals, a reaction with the fats or the fatty acids of the red cells might be the basis of the damaging (if it be so regarded) influence of the magnesium. No evidence, however, of such reaction has been observed, and the titrations with calcium, barium and manganese help to dispose of this hypothesis. Tests have been made to determine whether or not there is a preliminary reaction between the magnesium and the red cells, permitting hemolysis to proceed after the removal of the magnesium to the full extent as would be the case if the latter had a chemical effect on the cells themselves. To test this point, sheep cells were washed and stored in magnesium saline (magnesium chloride or sulphate 0.08 sodium chloride 8.42, distilled water 1000). Such suspensions were centrifuged the saline poured off, and the volume restored with plain distilled water saline. Titrations conducted with cells so treated showed no increased titer of hemolysin and therefore no evidence of a separate influence, damaging or otherwise on the red cells. Cells were also washed and suspended in both plain distilled water saline and magnesium saline and stored in the ice box. No difference was noted in the permanence of the cells.

Since the proof of the pudding is in the eating, and in the present case we must continue for a while along empirical lines, we add a representative

CONCLUSIONS

1 The calcium content of normal serum (10.7 to 13.2 mg per 100 cc) was found to be definitely higher than the value usually accepted (9 to 11 mg per 100 cc)

2 Of 50 normal individuals 7 per cent had a serum calcium content between 10.0 and 10.9 mg per 100 cc, 67 per cent between 11.0 and 11.9 mg per 100 cc, 24 per cent between 12.0 and 12.9 mg per 100 cc and 2 per cent above 13.0 mg per 100 cc

3 Ninety-one per cent of these cases had between 11.0 and 12.9 mg of calcium per 100 cc of serum, while only 7 per cent had less than 11.0 mg and none less than 10.0 mg

REFERENCE

¹Kramer, B., and Tisdall, F. F. A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, *Jour Biol Chem*, 1921, *xlvi*, 475

THE EFFECT OF PARAFFIN AND OILY SUBSTANCES UPON FILTER CANDLES

By W. L. HOLMAN, M.D., TORONTO, CANADA, AND F. M. KROCK, M.D.,
BALTIMORE, MD

A STUDY of bacterial filtration, started some years ago at Stanford University and later at Johns Hopkins University, is being continued by one of us (W. L. H.). The purpose of this paper is to call the attention of bacteriologic workers to a chance of error and to a possible new technique in filtration studies.

Our attention was first called to the effect of paraffin on filter candles while we were testing them by the method we have reported¹. One of the candles showed the escape of air bubbles from one spot on the candle at a pressure very much below that found in a previous test. We were for some time at a loss to discover what had happened to produce this spot. Finally we concluded after trying a number of treatments that the candle had probably come in contact on the laboratory table with a little paraffin, used for sealing tubes, which had melted in sterilizing the candle and had penetrated the pores.

This filter candle, a new Berkefeld V-3, required an air pressure of 565 mm of mercury before leakage occurred. After it had been accidentally "oiled" presumably by contact with paraffin on one spot (which showed white when immersed in water) and was being retested, air bubbles came through this white area at less than 200 mm pressure. The filter was then placed in xylol for forty-eight hours and allowed to dry, the white spot was no longer visible when placed in water, and the test showed 500 mm mercury pressure before air bubbles came through.

¹From the Department of Pathology and Bacteriology, University of Toronto and the Department of Pathology and Bacteriology, Johns Hopkins Medical School.
Received for publication February 11, 1926.

After this preliminary finding, several other filters were studied and various estimations were made as to the size of the pores, the space occupied in the dry candles by air, the amount of water absorbed, the capillary pressure the rate at which water or a peptone solution passed through, and the time under various filtration pressures at which they became permeable to bacteria. Having obtained certain standards we attempted to "oil" the filters by different methods. We tried petrolatum, paraffin oil and mixtures of the two. It was difficult not to get too much of the material into the filter pores, and we found they tended to become plugged. In attempting to clean them with ether, chloroform, by boiling and other methods, the partly cleaned filters would sometimes allow the passage of bacteria which were held back by the filter before and after treatment. A mixture of equal parts petrolatum and paraffin oil was used in most of the experiments on oiling the filters, but in the further studies of this problem it is planned to apply petrolatum, paraffin, and similar substances in solution using a volatile solvent such as ether or xylol.

Without, at this time giving the results of the estimations used in determining certain physical characteristics of the filters tested, the results obtained in filtration experiments will be given briefly. It should be mentioned, however, that our results confirm those obtained by Mudd in that the rate of filtration is of more value in determining the efficiency of filters than the estimation of the diameters of the pore spaces. The formula given by Bechhold for this latter purpose gives a higher value for the intergranular diameters, as the air pressure showing bubbles decreases. In our experiments the bubbles first appeared in the areas where presumably the spaces were partly filled with petrolatum, but the Bechhold formula applied here would indicate larger spaces. Mudd found the same type of results for old filters contaminated with dyes and proteins. The difference between our experimental results and those of others is to be seen in the fact that the "oiling" of the filters, although it cuts down the flow of fluid, makes the filters more permeable for bacteria. The ordinary plugged filter is as a rule less permeable for fluid and solids (including bacteria).

A motile culture of *B. prodigiosus* showing no viscid characters on media, was grown for twenty four hours at room temperature in large flasks of Dunham's peptone solution. Only twenty four hour cultures were used throughout, and the medium showed only a very faint cloud at this period. The apparatus employed enabled the removal of the filtrate at stated intervals, and all of the filtrate was used to test for the presence of bacteria, the collecting tubes, after being disconnected, were simply left at room temperature.

In a personal communication from Stuart Mudd he kindly called my attention to the fact that the Bechhold formula as used by him (*Jour. Bacteriol.* 1923, vol. 459) contains an important error and he has asked me to say that the estimated intergranular spaces given in his paper are ten times too small. The formula as given by Bechhold and used by Mudd was as follows: $D = \frac{4B \times 760}{p \times 1033} \times 10^6$. Bigelow and Bartell showed (*Jour. Am. Chem. Soc.* 1909, xxxi, 1194) that the last term in the denominator should have been 10, not 1033. Bechhold has acknowledged the mistake which will be corrected in the next edition of his book. This makes the intergranular diameters of the clean filters of all three types of the order of 4μ . This correction emphasizes the importance of the adsorption phenomena since most of the common bacteria are well under 4μ in diameter. Probably the filters owe their usual tightness to three things: (1) to the tortuosity of the channels through them, (2) to prompt reduction of the intergranular spaces by adsorption of material from the filtering fluid, (3) to adsorption of the bacteria themselves in the filter. Perhaps there are other causes also.

ture for a week or more to allow growth to take place. Since only a slight opalescence was evident in the peptone solution at the time of filtration and since this microbe will after a few days develop a very opaque cloud and scum in this medium, it was felt that this was a safer procedure than if we transferred the filtrate to fresh tubes of medium. The bacteria in this early stage of growth are rapidly dividing and are probably much smaller in size than later when their dividing activity has become more sluggish. This method, we believe, puts the candles to a very rigid test.

Investigators frequently use cultures of *B. prodigiosus* without, as a rule, specifying the stage of growth, the medium used, or the physical condition of the bacteria. These are of great importance in filtration studies. We³ have previously reported the successful filtration of a small anaerobic bacterium, resembling in morphology *B. pneumosintes*, through Mandler and Berkefeld N and W filters. This passage only occurred when the bacteria were grown in cooked meat medium without any addition of peptone or other nutrient material, and it failed to occur when the culture was taken from media containing peptone (Veillon agar, liver peptone broth). We consider that many of the bacteria are actually smaller under the conditions mentioned as they appear to be when examined microscopically.

The size of bacteria varies greatly not only from the effect of the available food but also with the stage of growth. Olitsky and Gates in a number of papers record successful filtration experiments with *B. pneumosintes* from nasopharyngeal washings in the first thirty-six hours of uncomplicated influenza and from a number of cultures presumably grown in ascitic-fluid rabbit-kidney medium with a petrolatum seal. Their paper⁴ on the filterability of their organism gives no details of their method of filtration. They used Berkefeld V and N candles, but the culture used for testing these is not mentioned. In two of their animal passages, however, using filtered material, *B. pyocyaneus* was found in the lungs of the treated animals. This, they explained, was due to faulty technique in filtration in that the Berkefeld candle had been contaminated with this organism. We presumed from this that *B. pyocyaneus* was used as the test culture, and we, therefore, also used it in our experiments. In 1922⁵ they reported striking morphologic changes of their bacterium when cultivated in dextrose peptone broth in that it became decidedly larger and more bacillary. The bacteria, however, reverted to the original minute form on cultivation in a dialysate of ascitic-fluid-rabbit-kidney medium. They did not give any filtration experiments on these cultures of larger forms, but it is presumed they would have been negative.

Mudd² in a very valuable contribution to the problems of filtration unfortunately used for controlling the filters a strain of *B. prodigiosus* which showed very pronounced sticky characters. He very kindly sent us his culture, and it corresponded to a very sticky strain which we had discarded as unsuitable for such a purpose. Hall and Howitt^{6, 7} recently failed in numerous tests on the filterability of the minute anaerobe above mentioned. They used 2 of our strains and 24 of their own. Their procedure was different from ours in many details. The test culture *B. prodigiosus* was included in the material filtered, but since this organism is considerably larger than the little anaerobe

and if it were present in quantity, it would undoubtedly plug the filter pores. They used only 20 cc for each candle while we used highly diluted saline suspension in large amounts to prevent the plugging of the surface pores of the candles. Tests were made for bacteria using 1 cc of the filtrate which varied from 2 cc to 19 cc, but we used the total filtrate collected at different intervals. The high pressure 637.5 mm used by them would tend to stop the filters, especially if many bacteria are present in the fluid. We used pressures varying from 100 mm to 400 mm. The time used (five minutes) is less than we used. We were particularly interested in comparing our organism with *B. pneumosintes*, and having no criteria from the reports of Ohitsky and Gates as to control culture, dilution of material, pressure used, time of filtration or quantity of filtrate tested, we tried altering these various factors with the result that we obtained passage of our minute anaerobe through candles impermeable to cultures of *B. proteanans*. This minute anaerobe is undoubtedly very close to the limits of filterability, and slight changes in size of the organism and conditions of filtration will affect the result. If size can be neglected, then most of the filtration experiments are absurd.

EXPERIMENTS WITH "OILED" FILTERS

We encountered some difficulty in the early attempts to remove the added petrolatum or oil. Mandler candle (2½ in.) No. 2, for example, which gave sterile filtrates after ten minutes at 100 mm plus eight minutes at 200 mm plus two minutes at 400 mm of mercury with a total of 64.5 cc of medium, after having been cleaned, dried and immersed in paraffin oil at 53° C for twenty-four hours, had the excess oil forced out by air pressure, was then autoclaved and tested with the above culture. The bacteria came through after a combined time of ten minutes at 200 mm and twenty-five minutes at 400 mm of mercury with 85 cc of the medium. The candle was then "cleaned" by passing ether through it for some time, thoroughly scrubbed with a brush, boiled, kept overnight in chloroform, again boiled for two hours and finally autoclaved. It was tested with a twenty-four hour culture, and the bacteria came through after ten minutes at 200 mm pressure in 11 cc of medium, and at a pressure of 400 mm, 47 cc of medium which contained bacteria and showed an oily film on its surface was collected in four minutes.

To overcome this trouble it was thought possible to burn out the oil and petrolatum in a Wiesnegg furnace. The results were disastrous, for after this treatment a number of the candles were cracked and the others showed loose collars. The preliminary tests on these candles showed, however, that the bacteria passed "oiled" and partially "cleaned" filters more readily than they did untreated candles. A number of examples will illustrate the results obtained.

Mandler candle (2½ in.) No. 4 which when new had withstood 100 mm pressure for ten minutes plus 200 mm for ten minutes plus 400 mm Hg pressure for seven minutes with a total filtrate of 67 cc was treated on one spot about a square centimeter in size with vaseline, was autoclaved and tested. Bacteria came through after five minutes at 100 mm plus five minutes at 400 mm mercury with 92 cc of medium. It was "cleaned" with alcohol and ether and gave bacteria under the same conditions in 80 cc of medium.

A further attempt at cleaning with ether, chloroform and prolonged boiling only resulted in reducing the amount filtered (and showing bacteria) to 75 cc in eight minutes.

Mandler candle ($2\frac{1}{2}$ in) No 5, gave 58 cc of filtrate free from bacteria after seven minutes at 100 mm pressure plus seven minutes at 400 mm mercury pressure. After treating with melted petrolatum, it was found almost plugged and required a 400 mm Hg pressure and a little heat to start it running. Bacteria were present in the 22 cc of the filtrate collected in fifteen minutes. After treatment with ether, chloroform, prolonged boiling and autoclaving, it gave 137 cc of sterile filtrate after twelve minutes at a pressure of 400 mm Hg. We considered this candle to have been at this time free from the petrolatum (which it probably was), but it was heated in the furnace and broken.

Mandler candle ($2\frac{1}{2}$ in) No 7 was tested before treatment, and it gave 70.5 cc of bacterial free filtrate after eight minutes at 100 mm plus eight minutes at 400 mm mercury pressure. One side of the candle was coated with petrolatum, autoclaved and tested. Bacteria came through in 15 cc of the medium after four minutes at 200 mm pressure. It was "cleared" in alcohol and ether and was retested. The bacteria grew in 35 cc of the filtrate obtained after five minutes at 200 mm pressure. A further attempt at cleaning with ether, chloroform, and prolonged boiling resulted in some removal of the petrolatum. The test gave 84 cc sterile filtrate after four minutes at 200 mm pressure plus three minutes at 400 mm, but the next 50 cc of filtrate after eight minutes at 400 mm pressure contained bacteria.

Mandler candle ($2\frac{1}{2}$ in) No 8 gave before treatment 56.5 cc sterile filtrate after five minutes at 100 mm plus five minutes at 400 mm pressure. After twenty-four hours immersion in a mixture of equal parts petrolatum and paraffin oil, it was found to be almost plugged and required thirty minutes to obtain 75 cc of filtrate at a mercury pressure of 400 mm, but this contained bacteria. This candle after the ether, chloroform, and boiling treatment gave 142 cc of sterile filtrate after four minutes at 200 mm plus six minutes at 400 mm mercury pressure. This would indicate that it was practically clear of the oily substances, but the preliminary test had not determined what point of time or pressure or what amount of filtrate would allow bacteria to pass through it.

The results of the various tests on these 4 filters are tabulated for comparison in Table I. Three Berkefeld V candles were tested, but since they rapidly gave bacteria in the filtrates before treatment, they were not further studied. Two of the Mandler candles (No 3 and No 6) which had escaped the furnace were further studied in Tables II and III. It will be seen that these candles are apparently effective in holding back bacteria under the conditions used. Tables IV and V show that at a constant pressure bacteria pass through after ten to fifteen minutes with both candles. With candle No 3 the amount of sterile filtrate reached 59 cc and with candle No 6, 74.5 cc while the filtrate containing bacteria was between these amounts and for candle No 3, 85 cc and for candle No 6, 102.5 cc. It will be seen that these amounts differ from those shown in Tables II and III where the time and the pressure were variable.

Since it is believed that higher pressures may sometimes prevent bacteria from passing a filter which they go through under lower pressure, it was thought advisable to try higher pressures. The two filters were thoroughly cleaned before testing further. This was done by repeated washings with water, boiling for one-half hour in a 2 per cent washing soda solution, boiling in water for one hour with frequent changes of the water, washing by passing water through the filter and by scrubbing with a brush. The filters were then autoclaved and tested.

TABLE I
VARIOUS TESTS WITH 2¹/₂ INCH MANDREL FILTERS

FILTER NUMBER	TREATMENT	PRESSURE MM HG	TIME IN MINUTES	RATE PER MINUTE	AMOUNT IN CC	RESULT*
4	New	100	10	27.5	275	0
4	"	200	10	18	180	0
4	"	400	7	215	215	0
4	"	-	27	-	670	0
4	One small spot treated with petrolatum	200	5	80	400	0
4	" " " " " "	400	5	104	520	+
4	" " " " " "	-	10	-	920	+
4	Water alcohol, ether Spot still seen	200	5	70	350	0
4	" " " " " "	400	5	90	450	+
4	" " " " " "	-	10	-	800	+
4	" " " " " "	400	10	35	350	+
4	" " " " " "	-	20	-	1150	+
4	Ether, boiled in water, CHCl ₃ overnight	200	5	50	250	0
4	boiled in water two hours	400	7	166	500	+
4	" " " " " "	-	8	-	750	+
5	New	100	7	228	230	0
5	"	400	7	50	350	0
5	"	-	14	-	580	0
5	Melted petrolatum Almost plugged	400				
		Warmed to start flow				
			15	140	220	+
5	" " " "	400	15	266	400	+
5	" " " "	400	15	100	150	+
5	Ether, CHCl ₃ and boiling	400	3	160	480	0
5	" " " " "	400	3	130	390	0
5	" " " " "	400	6	82	500	0
5	" " " " "	-	12	-	1370	0
7	New	100	8	37	295	0
7	"	400	8	51	410	0
7	"	-	16	-	705	0
7	One side with petrolatum	200	4	37	150	+
7	" " " " "	400	11	-		
				broken		
7	" " " " "	400	9	13	120	+
7	Water alcohol ether	200	5	70	350	+
7	" " " " "	400	10	55	550	+
7	" " " " "	400	10	25	250	+
7	Ether CHCl ₃ and boiling	200	4	105	420	0
7	" " " " "	400	3	140	420	0
7	" " " " "	-	7	-	840	0
7	" " " " "	400	8	625	500	+
7	" " " " "	-	15	-	1340	+
7	" " " " "	400	0	39	350	+
8	New	100	5	41	205	0
8	"	400	5	72	360	0
8	Petrolatum and paraffin oil	400	20	21	420	0
8	" " " " "	400	10	33	330	+
8	" " " " "	-	20	-	750	+
8	" " " " "	400	10	18	180	+
8	Ether CHCl ₃ and boiling	200	4	115	460	0
8	" " " " "	400	7	100	500	0
8	" " " " "	400	3	153	460	+
8	" " " " "	-	10	-	1420	+

+ = Bacteria in filtrate 0 = No bacteria in filtrate.

TABLE II

MANDLER CANDLE No 3

RESULTS OF FILTERING A TWENTY FOUR HOUR CULTURE OF B PRODIGIOSUS AT DIFFERENT PRESSURES

Time in minutes	10	10	12
Rate per minute in c c	2 2	2 05	2 166
Total in c c for each period	22	20 5	26 0
Total of fluid filtered in c c	22	42 5	68 5
Pressure in mm mercury	100	200	400
Result	0	0	0

TABLE III

MANDLER CANDLE No 6

RESULTS OF FILTERING A TWENTY FOUR HOUR CULTURE OF B PRODIGIOSUS AT DIFFERENT PRESSURES

Time in minutes	7	7
Rate per minute in c c	3 4	4 3
Total in c c	24 0	30 0
Pressure in mm mercury	100	400
Result	0	0

TABLE IV

MANDLER CANDLE No 3

RESULTS OF FILTERING B PRODIGIOSUS AT A PRESSURE OF 200 MM MERCURY

Time in minutes	5	5	5	5	5	5	5	5	10	5*
Rate per minute in c c	6 2	5 6	5 2	4 6	3 6	3 0	2 7	2 4	2 2	2 6
Total in c c for each period	31 0	28 0	26 0	23 0	18 0	15 0	13 5	12 0	22 0	13 0
Total of fluid filtered in c c	31 0	59 0	85 0	108 0	126 0	141 0	154 5	166 5	188 5	201 5
Result	0	0	+	+	+	+	+	+	+	+

*Pressure 400 mm mercury

TABLE V

MANDLER CANDLE No 6

RESULTS OF FILTERING B PRODIGIOSUS AT A PRESSURE OF 200 MM MERCURY

Time in minutes	5	5	5	5	5	5	5	5	10	5*
Rate per minute in c c	7 8	7 1	5 6	4 2	3 6	3 1	2 6	2 2	2 0	2 6
Total in c c for each period	39 0	35 5	28 0	21 0	18 0	15 5	13 0	11 0	20 0	13 0
Total of fluid filtered in c c	39 0	74 5	102 5	123 5	141 5	157 0	170 0	181 0	201 0	214 0
Result	0	0	+	+	+	+	+	+	+	+

*Pressure 400 mm mercury

Candle No 3 was tested with a pressure of 650 mm of mercury, and it will be seen no bacteria came through in 80 c c of medium in fifteen minutes. Candle No 6 under a pressure of 200 mm of mercury did not allow the passage of any bacteria in 98 c c of filtrate in fifteen minutes. It is suggested that the cleaner walls account for the difference between the results shown in Tables IV and V and those shown in Tables VI and VII. With these results as a basis, the two filters were then treated, after cleaning and drying, with a mixture of equal parts of petrolatum and paraffin oil, autoclaved and tested (using a constant pressure of 200 mm of mercury). The results are shown in Tables VIII and IX.

The 2 candles permit the passage of bacteria in between ten and fifteen minutes filtration and an amount of between 225 c c and 305 c c for candle No 3 and between 149 c c and 189 c c for candle No 6. It is to be noted that the rate of filtration is much slower than in Table VII under the same pressure. The petrolatum-oil mixture tends to fill the passages, but

TABLE XI

MANDLER CANDLE No 6

RESULTS OF FILTERING B PRODIGIOSUS AFTER CANDLE HAD BEEN TREATED IN XYLOL FOR TWENTY FOUR HOURS, IN ANTHORMIN FOR TWENTY FOUR HOURS, WITH DISTILLED WATER PASSING THROUGH FOR ABOUT FIFTEEN HOURS, BOILED AND AUTOCLAVED PRESSURE 200 MM MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c c	100	93	87	80	75	69	60	55	45	40	35	325
Total in c c	100	193	280	360	435	504	564	619	664	704	739	869
Result	0	0	0	0	0	0	0	0	0	0	0	0

TABLE XII

MANDLER CANDLE No 3

RESULT OF FILTERING B PRODIGIOSUS AFTER CLEANING AND "OILING" CANDLE AS IN TABLE VIII PRESSURE 200 MM MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c c	20	30	30	30	30	30	30	25	25	25	25	18
Total in c c	20	50	80	110	140	170	200	225	250	275	300	372
Result	0	0	0	0	0	0	0	0	0	+	+	+

TABLE XIII

MANDLER CANDLE No 6

RESULT OF FILTERING B PRODIGIOSUS AFTER CLEANING AND "OILING" CANDLE AS IN TABLE IX PRESSURE 200 MM MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c c	40	40	40	35	35	25	30	30	25	25	20	125
Total in c c	40	80	120	155	190	225	255	285	310	335	355	405
Result	0	0	0	0	0	0	0	0	0	0	0	+

TABLE XIV

MANDLER CANDLE No 3

RESULT OF FILTERING B PRODIGIOSUS AFTER CLEANING CANDLE WITH XYLOL FOR TWENTY FOUR HOURS NO ANTIHORMIN PRESSURE 200 MM MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c c	40	60	70	70	70	70	70	70	70	70	70	675
Total in c c	40	100	170	240	310	380	450	520	590	660	730	1000
Result	0	0	0	0	0	0	0	+	+	+	+	+

TABLE XV

MANDLER CANDLE No 6

RESULT OF FILTERING B PRODIGIOSUS AFTER CLEANING CANDLE WITH XYLOL FOR TWENTY FOUR HOURS NO ANTIHORMIN PRESSURE 200 MM MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c c	60	80	80	80	80	80	80	80	70	70	70	65
Total in c c	60	140	220	300	380	460	540	620	690	760	830	1090
Result	0	0	0	0	0	0	0	0	0	0	+	+

There are two indications in Tables X and XI that the filters have been freed of the mixture. First the medium comes through very much more quickly so that with No 3, 738 c c is filtered in fifteen minutes in contrast to only 305 c c in the oiled candle (Table VIII) and with candle No 6, 869 c c in contrast to 189 c c in the oiled filter (Table IX). Second no bacteria came through although 24 and nearly 46 as much medium was obtained in the same time as in the oiled filters. The results shown with clean filters in Tables VI and VII should also be compared. After thorough washing and

drying the two candles were treated as before with the petrolatum oil mixture autoclaved and tested

In Tables XII and XIII it is seen that the filtration is slower and that bacteria pass through within the time limit of fifteen minutes. With candle No 3 this occurred when 27.5 c.c. had filtered in ten minutes and with candle No 6 after 40.5 c.c. had been collected in fifteen minutes. The results are in keeping with those shown in Tables VIII and IX although it would appear that there was more plugging of the spaces in the results tabulated in Table VIII than in Table XIII and a trace less in candle No 3 of Table XII. The filters were again treated with xylol for twenty four hours but not with antiformin, autoclaved and tested.

Much more fluid came through both candles than in the previous experiments with "clean" filters and bacteria passed the filter within the fifteen minutes of the test. It is to be noted however that with filter No 3 (Table XIV) it required 52 c.c. before bacteria were found in contrast to the other tests on oiled filters viz., 30.5 c.c. (Table VIII) and 27.5 c.c. (Table XII). It is true it only needed eight minutes to filter this 52 c.c. With filter No 6 83 c.c. containing bacteria came through in ten minutes other experiments with this candle when 'oiled' show a rate of 18.9 c.c. in fifteen minutes (Table IX) and 40.5 c.c. in fifteen minutes (Table XIII) with bacteria. It is believed, nevertheless, that our treatment with xylol alone did not completely remove the oily mixture from the filter.

This work is being continued and a new series of candles is being carefully measured to determine the pore space so that it can be estimated how much of this is filled with the petrolatum paraffin oil or paraffin when the dry candles are treated with these substances in solution in ether, xylol, benzene or other solvent. Measurements are also being collected on the capillary pressure, the amount of water absorbed and the rates of flow for different fluids so that the alterations resulting from the oiling may be more accurately determined.

The object of this research is to enable us to remove the adsorptive character of the surface walls of the intergranular spaces without interfering too greatly with the rate of filtration. It is well known that filtration through clay or porcelain candles does not result in merely a mechanical separation of bacteria due to their failure to pass the small interstices of the candle but depends on the action of complicated physical and chemical adsorption and other laws, and it is in an endeavor to lessen this adsorptive character that this study is being followed.

In filtering all manner of substances the loss of valuable constituents by their adhesion to the filter wall has been considered inevitable. The loss of antitoxin from antitoxic serums, the disappearance of enzymes which are supposed to be present in a fluid, the complete absence of virus after filtration experiments shown by testing with ground up filter to be in the filter substance, and the many other examples of similar phenomena make the development of a better method of filtration highly desirable. It is to be hoped when the technique for oiling the candles is made more perfect that some information may be obtained in filtration studies of smallpox and other viruses.

There is another equally important side to the problem and that is the danger involved in accidentally bringing petrolatum or other oily substance in contact with filter candles. If this occurs between the test for its efficiency and the actual filtration, the intermediate autoclaving causes a distribution of the oily substance over the intergranular surfaces of the candle and may lead to erroneous results. This is a danger to be avoided in the filtration of anaerobic cultures where petrolatum is employed on the surface of fluid media. Since petrolatum and paraffin are so widely used in laboratories for lubricating purposes, the chances of this accident occurring are multiplied.

CONCLUSIONS

1 There is a potential danger present in many laboratories of accidentally changing the character of filter candles by permitting them to come in contact with small amounts of petrolatum, paraffin and similar substances.

2 These oily substances render the candles more permeable to bacteria and somewhat less permeable to fluids. Air bubbles also pass such filters under lessened pressure.

3 Autoclaving and ordinary methods of cleaning do not remove such substances, and an active solvent, such as xylol, is necessary.

4 Oiled filter candles may be useful in the study of many materials where the loss of important constituents by adsorption to the filter walls is to be avoided.

5 The application of the Bechhold formula in determining the size of the pores of filters would make it appear that these are larger after treatment with oily substances when actually they must be smaller. It is useless, therefore, in determining the efficiency of such treated filters.

REFERENCES

- ¹Krock, F. H., and Holman, W. L. A Simple Apparatus for Testing and Washing Filter Candles, *Jour. Am. Med. Assn.*, 1923, lxxx, 1142.
- ²Mudd, S. The Penetration of Bacteria through Capillary Spaces. I. Motility and Size as Influencing Filterability through Berkefeld Candles, *Jour. Bacteriol.*, viii, 1923, 459.
- ³Holman, W. L., and Krock, F. H. Microorganisms Suggesting B. Pneumointes from the Mouths of Man and Laboratory Animals, *Am. Jour. Hyg.*, 1923, iii, 487.
- ⁴Olitsky, P. K., and Gates, F. L. Experimental Studies of the Nasopharyngeal Secretions from Influenza Patients. II. Filterability and Resistance to Glycerol, *Jour. Exper. Med.*, 1921, xxxiii, 361.
- ⁵Olitsky, P. K., and Gates, F. L. Experimental Studies of the Nasopharyngeal Secretions from Influenza Patients. VIII. Further Observations on the Cultural and Morphological Characters of Bacterium Pneumointes, *Jour. Exper. Med.*, 1922, xxxv, 813.
- ⁶Hall, I. C., and Howitt, B. The Anaerobic Bacteria of the Oral Cavity, *Proc. Soc. Exper. Biol. and Med.*, 1925, xxii, 541.
- ⁷Hall, I. C., and Howitt, B. Bacterial Factors in Pyorrhea Alveolaris. IV. Micrococcus Gazogenes, A Minute Gram Negative Nonsporulating Anaerobe Prevalent in Human Saliva, *Jour. Infect. Dis.*, 1925, xxxvii, 112.

THE STANDARDIZATION OF TUBERCULIN*

By JOSEPH D. ARONSON, M.D. PHILADELPHIA, PA

TO determine the value of the methods proposed for standardizing tuberculin, the strength of 10 samples of tuberculin was ascertained by means of the intradermic method the complement fixation and the precipitin reaction. These results were compared with those obtained by the Bureau of Animal Industry employing the toxic dose of tuberculin as the criterion of potency.

The intradermic standardization was carried out on guinea pigs infected intraperitoneally, four weeks previously with a human strain of *B. tuberculosis*. The same dose of 4 different samples was injected intradermally at widely separate parts of the abdomen. The doses employed were 0.01, 0.001, 0.0001 c.c., diluted with normal saline to a volume of 0.1 c.c. Forty-eight hours later the results were read and recorded as A, B, C or D depending upon the degree of necrosis, edema or redness.

To determine the strength of tuberculin by means of the precipitin reaction, amounts of tuberculin ranging from 0.02 c.c. to 0.0000005 c.c. were added to 0.2 c.c. of an immune serum prepared by repeatedly injecting a goat intravenously with a living avirulent strain of *h. tuberculosis* bacillus. After four hours incubation the results were read the next day and recorded as XXXX, XXX, XX, X, trace or negative.

The complement fixation reaction was carried out by adding varying amounts of the different samples of tuberculin to a fixed amount of the same immune serum used in the precipitin reaction. After adding two units of fresh guinea pig complement and incubating the tubes for two hours, sheep cells and anti-sheep amboceptor were added and the final results were read the next day.

A comparison of the results obtained by the different methods indicates that a close agreement exists between the results obtained with the intradermic method and that obtained by determining the toxic dose. On the other hand, the results obtained by means of the complement fixation or the precipitin reaction do not agree with those obtained with the first two methods.

This study indicates that the intradermic method can be utilized to standardize tuberculin and we suggest that a tuberculin to be considered of standard strength should produce a definite edema and redness with 0.001 c.c. of the tuberculin.

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.
Received for publication February 28, 1926.

LABORATORY METHODS

A NEW TYPE OF MOTOR DRIVEN LONG PAPER KYMOGRAPH*

By D E JACKSON, PH D , M D , CINCINNATI, OHIO

THIS kymograph has been designed to meet all ordinary requirements in the way of a long paper kymograph not only for student use but also for the most exacting research work. A very large range of adjustments and a number of special, newly designed facilities are embodied in the construction of the kymograph.

The instrument is driven by a small motor which runs on either direct or alternating current of 110 volts, thus providing power continually so long as current is supplied. The kymograph is compact, sufficiently heavy to resist vibrations and is made of metal throughout. It is only about two thirds as large as the ordinary makes of long paper kymographs.

The main supporting stand (1) is made of heavy angle iron firmly held together by crossbars at the bottom and by two heavy iron plates (2 and 3) at the top. A crank (4) turns a worm which engages in the gear (5) which is mounted on a cylinder on which is wound the small steel cable (6) by means of which the main I-beam and the drums may be easily and quickly raised or lowered. The lower ends of the cable (one on either side) are attached to the lower end of the square main supporting shaft (23) on which is pivoted (at 7) the square iron block (24) to which is hinged (at 9) the main I-beam and also the semicircular, slotted plate by means of which lateral adjustment of the drums on a horizontal axis may be made in any position from horizontal to perpendicular on either side by means of the bolt (8) which is turned by a small rod passing through the bolt head. At (10) a rod passes through the head of a bolt which locks the main I-beam in any desired position as turned on a perpendicular axis.

The motor (11) by means of the belt (22) actuates a double series of worm and spur gears which greatly reduce the speed of the motor and give a series of ten changes in speed. The shifts from one to another of these changes can be quickly and easily made (while the motor is running if desired) by means of the two levers (14 and 15) and the lock knob (13). The belt (16) rides on two small cone pulleys which serve to double the ten rates of speed produced by the gears alone, thus making twenty rates of speed for the shaft on which is mounted the worm gear which turns the wheel (17) which, by means of a set screw, is adjustably mounted circularly on the shaft of the right-hand drum. By loosening the set screw the drum is thrown out of gear and can be easily

*From the Department of Pharmacology of the University of Cincinnati Medical School Cincinnati Ohio

Received for publication August 12 1926

and independently turned by means of the crank (19). In this way the paper, which is pasted around the two drums while they are turned down in a horizontal position, is smoked, thus avoiding the use of a special drum smoker. Tightening the above set-screw again throws the drum in gear and transmits (through the wheel 17) the full twenty different speeds to the drum. If it is especially desired two extra cone pulleys can be attached to the motor and to the main gear wheel drive shaft thus doubling the twenty previous speeds and

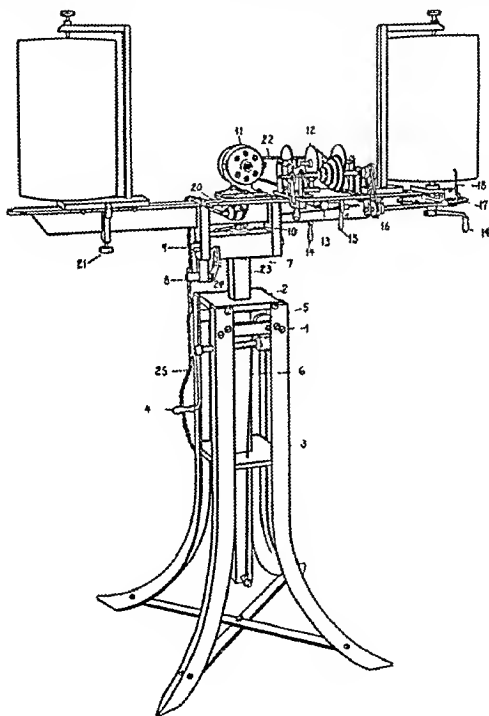


Fig. 1

giving forty speeds to the drum. It is also possible to attach a special mechanical spinning device to the right-hand drum if desired.

The switch (20) controls the current which reaches the motor through the extension cord (25) which attaches to any lamp socket.

At (18) is shown a special time marking device which is run directly by the main drum driving mechanism. This time marker is exceedingly convenient and can be readily raised or lowered or turned off the drum altogether at any moment desired. It is only as accurate as the speed of the motor which may

vary one per cent or more but in almost all instances is less liable to change and error than are other elements involved in the usual type of experiments performed. At any time the marker (18) can be turned off the drum and any other desired time recorder can be substituted. This change, however, I have never found it worth while to make in my own work.

The drums are full twelve inches high and eight inches in diameter. A record eight or nine feet long and one foot wide can thus be made. The position of the left-hand drum can be adjusted by set-screw (21).

The lock bolt (10) can be readily turned out entirely thus permitting the main I-beam and the drums to be lifted off of the main supporting stand.

Just below knob (13) and extending behind lever (15) is an extra long writing point which can be substituted for writing point (18), thus marking the time record up to the middle of the drum or higher in case two or more rounds of tracings are taken on the same long strip of paper (as would usually be the case).

This kymograph has been mainly constructed for me by Mr. George Grathwohl, mechanic in the department of pharmacology. The kymograph may be purchased from the Max Woehner & Son Company of Cincinnati, Ohio.

AN ADJUSTABLE SPHYGMOSCOPE FOR THE RECORDING SPHYGMOMANOMETER*

BY JOSEPH ERLANGER, M D, ST LOUIS, MO, AND W J MEEK, PH D, MADISON,
WIS

EVER since the difficulty first arose of obtaining suitable rubber bulbs for the sphygmomanometer devised by one of us,¹ and it became obvious that some other type of sphygmoscope would have to be developed if the instrument was to be kept available for further use, tests have been in progress in an effort to find a suitable substitute. While engaged in this search, one of us (Meek) found that a clamped-off segment of Gooch crucible tubing was satisfactory for the purpose. After trying out a variety of samples a form of rubber known in the trade as "band tubing" was finally found which not only is satisfactory but in addition permits of certain adaptations which make it superior in three respects to the rubber bulb originally employed. These advantages consist first, in the possibility of utilizing in the construction of the sphygmoscopic parts of the instrument, materials most of which are quite readily obtainable, if, indeed, they are not to be found on hand in many experimental laboratories, second, in being able, by means of a simple expedient, to adapt, within fairly wide limits, the sensitivity of the instrument to the pressure range of the subject under observation, and third, on account of this greater sensitivity, in being able to substitute for the compound recording lever with its relatively high inertia, a

*From the Physiological Laboratories of the Washington University School of Medicine and of the University of Wisconsin.

Received for publication July 9 1926

lever of simple construction and low inertia. This paper describes the means whereby these ends are accomplished and also certain modifications in the construction of the sphygmomanometer that have been made in this laboratory in recent years with a view to simplification of the adjustments necessary to adapt the instrument to changing and special conditions.

THE SPHYGMOSCOPE, SIMPLE FORM

Fig 1 is a diagram showing the new sphygmoscope in a form that can be put together in any laboratory. *A* is a piece of the band tubing (No 12, $1\frac{1}{2}$ inch, black rubber) 14 to 15 cm in length, which is drawn over a brass tube *B*, of such an outside diameter (0.97 inches) that the rubber *just is not stretched* by it. If this fit be too tight, so that more than 30 mm Hg of distending pressure becomes necessary to lift the rubber from the cylinder, it

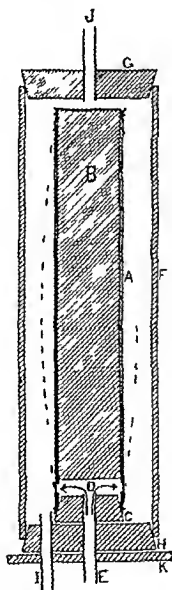


FIG 1.—The sphygmoscope laboratory construction. X $\frac{1}{2}$. Description in text.

will be impossible to determine low diastolic pressures. The rubber tube is securely fastened to the cylinder so as to make air tight the space between them. In order to keep down the dead space of the instrument the brass tube is filled with wax or other suitable material, leaving at one end however, a space deep enough to take a rubber stopper, *C*, and an air chamber, *D*, 5 to 8 mm in depth. Two holes about 3 mm in diameter (indicated by the arrows) are drilled through the brass tube into this air space. A hole bored through the stopper, *C*, carries the communication, *E*, between this air space,

and consequently between the space under the band tubing, and the remainder of the pressure space of the sphygmomanometer. The housing, *F*, of the sphygmoscope is a glass tube (so-called 2 inch) closed above and below by thin rubber stoppers, *G* and *H*. The lower stopper is provided with two holes through which the two tubes of the sphygmoscope base pass, one, *E*, into the inner or pressure chamber, the other, *I*, into the outer or tambour space. The upper stopper has one hole, *J*, through which communication is made with the recording tambour. To substitute this sphygmoscope for the one originally a part of the sphygmomanometer it is merely necessary to remove the tambour holder and the supporting frame on which it rests. The tambour can then

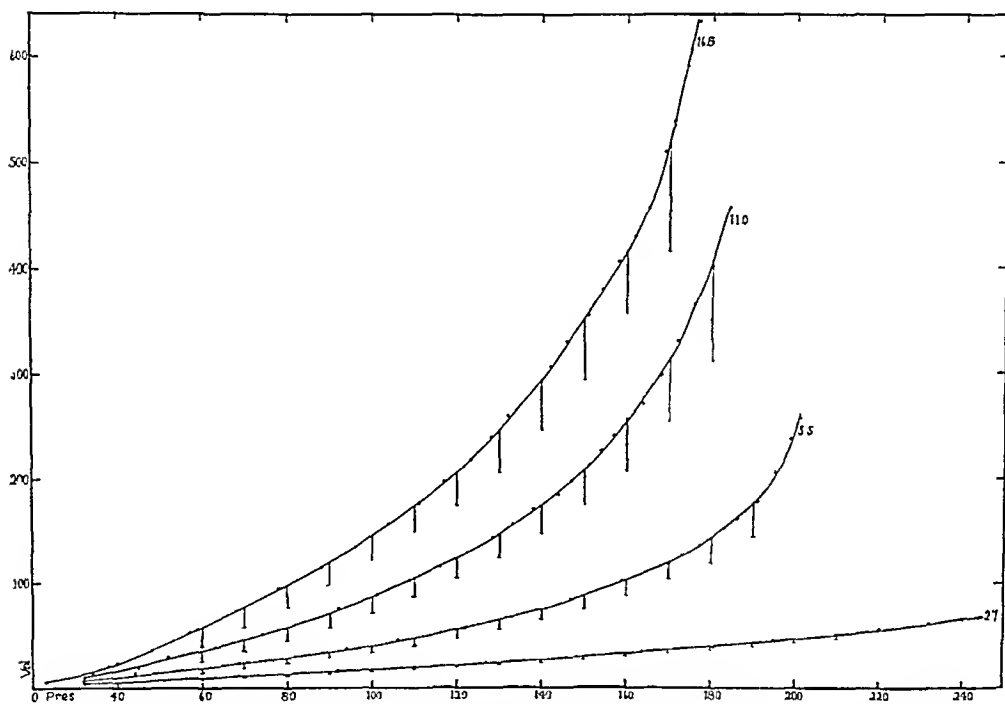


Fig. 2—Curves obtained by plotting the extensibility (ordinates) of four different lengths (27, 55, 110 and 168 cm.) of band tubing against the distending pressure (abscissae). For further description see text.

be supported on a separate stand or on a rod which any mechanic can erect on the base of the instrument. The sensitivity (extensibility) of the sphygmoscope can be altered to suit the case under observation by varying the length of band tubing exposed to the stretching pressure. This is determined by the distance of the upper ligature securing the rubber to the brass tube, from the lower.*

Various tests have been made in order to ascertain the capabilities of this new type of sphygmoscope. In the first place the extensibility has been determined of different lengths of band tubing tied as described above to the inserted brass tube. In these experiments the pressure distending the rubber tube was increased in steps and the volume of air displaced by the ballooning tube enclosed in its glass housing was measured by the move-

*A more convenient method of accomplishing this end is described below.

ment of a bead of water occluding the lumen of a horizontal glass tube of relatively narrow bore. Preceding each increment of pressure this bead was brought back to its initial position thus eliminating inequalities in the bore of the tube as a possible source of error. The results obtained with band tubing lengths of 27, 55, 110 and 168 cm are plotted in Fig 2, in which the horizontal axis gives the distending pressures in mm Hg the vertical axis the distentions of the rubber in terms of length of the horizontal tube used to measure the volume changes.

These curves indicate firstly the limiting pressures tubes of various length can sustain. The short st length 27 cm withstands a pressure of

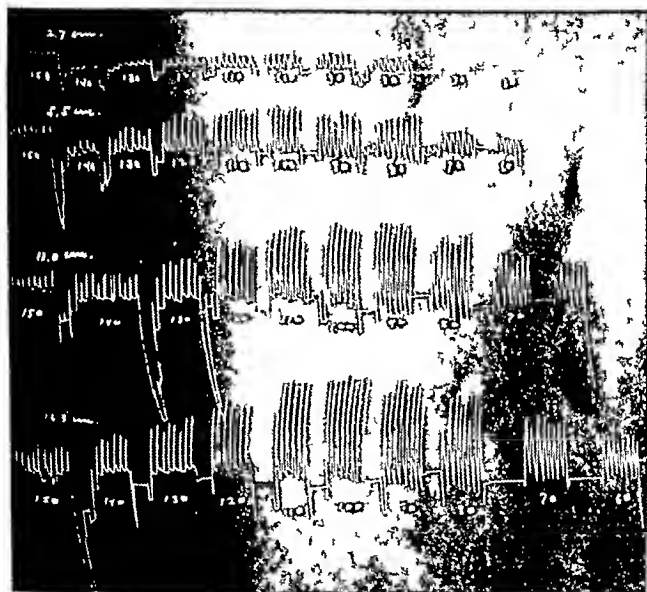


Fig 3—Records by the method of intermittent escapement obtained in rapid succession from one subject, with four different lengths (the same as in Fig 2) of rubber tube exposed namely 27, 55, 110 and 168 cm as indicated but otherwise under constant conditions

over 290 mm Hg (the limit of the manometer employed in making the tests was 260 mm Hg) whereas with the longest tube the limit of extensibility is rapidly approaching at a distending pressure of 174 mm Hg. Obviously the absolute values obtained will vary somewhat according to the properties of the particular specimen and the age of the rubber.

The curves indicate, secondly, that the volume oscillations produced by a given pressure oscillation will decrease as the basal pressure decreases. This behavior is visualized in Fig 2 by dropping from the curves at 10 mm Hg intervals perpendiculars equal in length to the volume change produced

by the corresponding 10 mm Hg pressure change. The inherent properties of the sphygmoscope, therefore, would tend (a) to counteract the increase in oscillation amplitude which is one of the signs of systolic compression and (b) to give a picture resembling the index to diastolic compression, namely, a decrease in the amplitude of the oscillations.

(a) Inasmuch, however, as the best index to the systolic pressure is the change in the form of the oscillations^{2,3} the type of extensibility exhibited by the sphygmoscope does not interfere with the reading of the systolic pressure.

(b) To be in a position to estimate the degree to which the reading of the diastolic pressure might be influenced by this property of the sphygmoscope it is necessary to have clearly in mind the oscillatory sign of diastolic compression. It is not, as is usually stated in the textbooks, the last of the highest oscillations. Neither is the diastolic pressure correctly marked by the first decrease in the size of the oscillations recorded during decompression. Rather, it is the point where the accelerating decrease changes into a retarding decrease in amplitude. To state this in another way, the sign is the point of inflection of the curve of amplitude decrease.⁴ This, presumably, is the same as the point selected by MacWilliam and Spencer⁵, namely, "just after the abrupt diminution" in oscillation amplitude. Now, a glance at the curves of extensibility of the band tubing (Fig 2) shows that though the vertical lines (oscillations) decrease in amplitude with decompression, there is nowhere a point of inflection in the curve of amplitude. It is also clear that any tendency on the part of this behavior of the sphygmoscope to obscure the diastolic sign could be minimized by using a length of band tubing that yields in the region of the diastolic pressure a nearly linear curve. The curve obtained with the 27 cm tube is practically linear up to pressures of 120 mm Hg, or more, that of the 55 cm tube up to 90 mm Hg whereas in the case of the 11 and 16.8 cm tubes the linear ranges are considerably more limited. When we are dealing with normal diastolic pressures, therefore, the properties of the band tubing, up to lengths of 55 cm, would not interfere appreciably with the diastolic sign, indeed, owing to the peculiar characteristics of the sign, no confusion could result even in the case of the longest of the segments.

To put to a practical test the questions raised by these considerations the sphygmoscope employed above was attached to a sphygmomanometer and records were obtained from one and the same individual, while maintaining constant all conditions excepting the length of band tubing. The lengths tested were those used in obtaining the data for the four curves reproduced in Fig 2. The records, shown in Fig 3, were made by the method of intermittent escapement. It can readily be made out by simple inspection, but much more clearly by plotting the amplitude of oscillation (average height of the oscillations at each compressing pressure) against the compressing pressure (Fig 4), that there is an inflection in the decline

*Hediger unaware of the previous work on this subject, redescrines this change in form as a sign of systolic compression. Neither does Hediger know that instruments with which continuous decompression records of the pulse can be made have been available for many years.

in amplitude that occurs during decompression. In three of the records this inflection (indicated by the arrows) appears between 80 and 70 mm Hg, in the fourth record it follows 70 mm.

The systolic sign, namely, the *first* clear increase in the spread of the limbs at the base of the pulse wave in each of the four records appears in the 120 mm series of oscillations, and is perfectly definite, except in the case of the record obtained through the medium of the shortest segment (27 cm), in which the oscillation amplitude is so small that details of form are obscured by the friction between the writing point and the paper. But even here the sign is not entirely effaced.

When these blood pressure records are considered in relation to the corresponding curves of extensibility of *this particular sample* of band tubing, it becomes perfectly evident that the 55 cm length is adequate for the

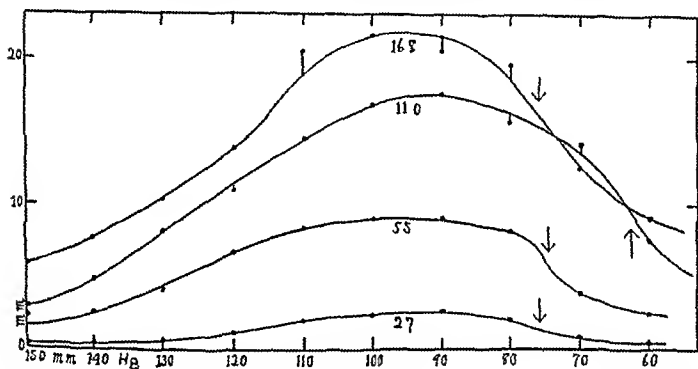


Fig. 4.—The diastolic sign in Fig. 3 elucidated by plotting the mean height of the oscillations at each compressing pressure (ordinates) against the compressing pressure (abscissae). The arrows mark the points of inflection of the amplitude decrease (the diastolic sign).

determination of normal arterial pressures. The oscillation amplitude it gives is quite sufficient, its extensibility covers a wide enough range (up to 180 or more mm Hg), and the diastolic oscillations are written in the range (up to 90 mm Hg), within which the extensibility curve of the rubber still is practically linear. A tube 10 to 12 cm long, it is seen, would under normal conditions give an oscillation that is higher than is really necessary. Furthermore, the extensibility of a tube of that length increases in such a way that pressures exceeding 160 mm Hg could not be satisfactorily measured with it. These characteristics, however, are just the ones that would facilitate the measurement of the arterial pressures in conditions of hypotension. Finally, for the determination of the arterial pressures in cases of hypertension it would be necessary to have a sphygmoscope that would record oscillations up to 250 to 300 mm Hg, this, the shortest (27 cm segment) would do. In hypertension cases, furthermore, the oscillation amplitude would probably be considerably higher than in the normal records.

shown in Fig 3 and Fig 7, for along with the increase in arterial pressure there usually is an increase in pulse pressure also

To record satisfactorily the oscillations transmitted by the rubber bulb sphygmoscope a compound lever was necessary, a simple lever suffices with

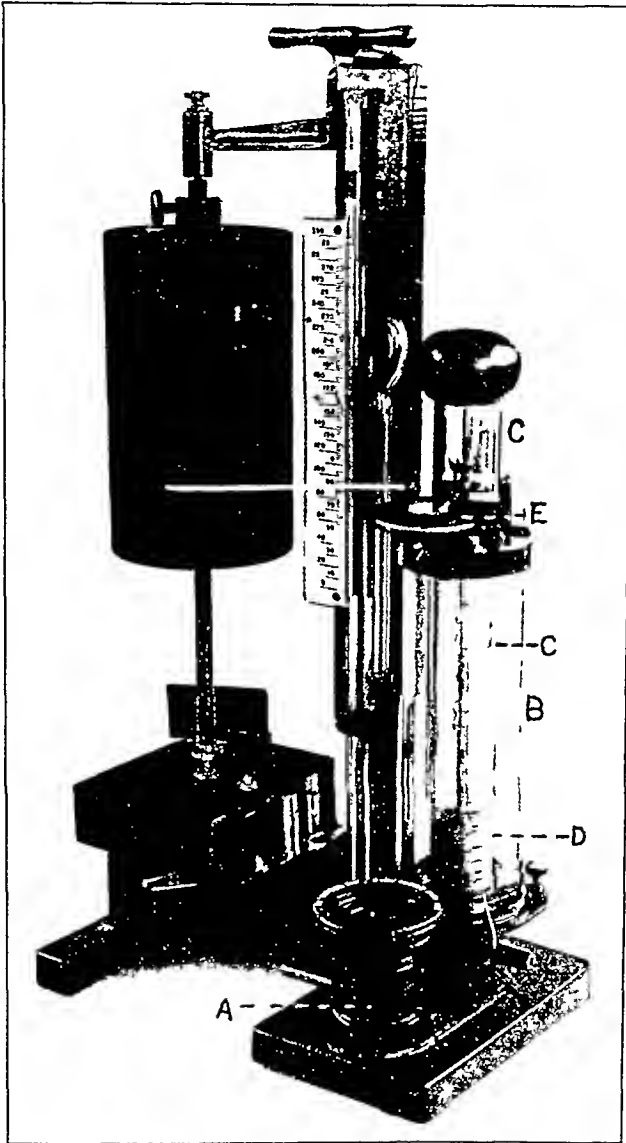


Fig 5—The sphygmomanometer as now constructed

A = compound stopcock

B = sphygmoscope

C = sliding sleeve for the adjustment of the sensitivity of the sphygmoscope

D = band tubing

E = tambour with simple lever

the present sphygmoscope, to which the records reproduced in Figs 3 and 7 bear witness. Not only is construction thus simplified but inertia also is diminished and consequently instrumental deformation of the recorded pulses

This sphygmoscope together with an arm band and pump form a convenient and accurate sphygmograph which can be used in connection with a polysphygmograph in the manner suggested by Halsey.*

THE SPHYGMO-COIL ON THE MODIFIED SPHYGMOMANOMETER

The foregoing considerations and observations clearly indicate the advantages that are to be gained by adapting the new form of sphygmoscope to the sphygmomanometer.* The manner in which this has been done is indicated in Fig 5 which pictures the entire sphygmomanometer as it is constructed in this laboratory. A detailed description of the instrument as a whole is unnecessary. Neither need a detailed description be given of the sphygmoscope since the method of putting together the simpler form described above, suffices to indicate the construction of the shop made form. It is necessary however to call attention here to the device that has been added to facilitate changing the length of band tubing exposed to the compressing pressure.

This is accomplished by a metal tube which slides over the band tubing from above and thus prevents the parts of the rubber covered by it from extending under the distending pressure. With inslated metal cylinder and band tubing exactly of the dimensions specified above a piece of Shelby steel tubing (C Fig 5) 6 inches long $1\frac{1}{8}$ inches in outside diameter and Number 16 gauge does perfectly for this purpose. The motion of the metal sleeve over the rubber tube is facilitated by keeping the surfaces covered with powdered soapstone and by advancing the sleeve with a spiral motion. To prevent leakage in the tambour space an accurate sliding fit between the steel tube and the brass top of the sphygmoscope is necessary.

It might be added that the recording tambour now connects directly with the sphygmoscope by means of a conical joint. It is therefore a simple matter to remove the tambour for the purpose of stretching its head or in order to record with it the oscillations upon another than the kymograph attached to sphygmomanometer.

Advantage is taken of this opportunity to describe also certain changes in the compound stopcock which though introduced some years ago have never been published. These alterations have been made in order to facilitate the determination of the pressures by the method of intermittent escape ment and also the adjustment of the capillary openings into the tambour and pressure spaces. Fig 6 gives the plan of the new stopcock. The designations are now on a dial attached to the plug of the stopcock and are *IN CLOSED INT SLOW CLOSED FAST* and *OUT*. As heretofore in position *IN* the pressure space can be inflated while the tambour head is protected by an opening connecting the tambour space freely with the exterior. At *CLOSED* the tambour and pressure spaces both are closed with the exception of a very minute leak in the former (now adjustable by means of a knurled screw under the tambour) provided in order to eliminate the effects

*The parts ready for attachment to instruments obtained in recent years from Schneider Brothers as well as the sphygmomanometer as a whole can be supplied by Mr John H Zimmer Mechanical Department Washington University School of Medicine to whom the authors are indebted for valuable assistance in working out many of the mechanical details.

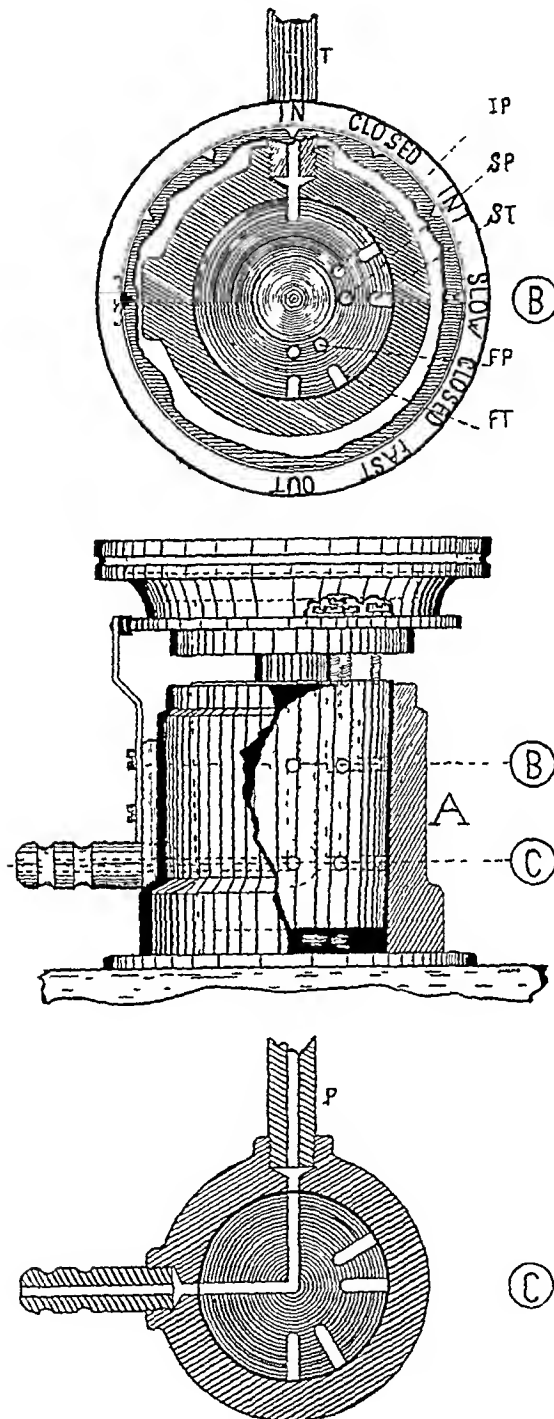


Fig 6—The compound stopcock. A, front view, B, section at the level of the tube T, connecting with tambour space C, section at the level of the tube P connecting with the pressure space. The designation that is aligned for reading from the front of the instrument (like IN, in the figure) indicates the operating position of the stopcock. The openings through the top of the plug are arranged on two circles those on the outer circle connecting with the tambour space through the upper tube T, those on the inner circle with the pressure space through the lower tube P, of these IP, SP, ST FP, and FT are adjustable by means of the screws shown in A. The openings operative in any given procedure are on the radius passing through the corresponding designation.

of venous engorgement and muscular movement on the general level of the lever. At *INT* (intermittent escapement) the compression diminishes at a rapid rate, controlled by the screw over the opening, *IP*, and at the same time the tambour space is in free communication with the exterior, and when the cock is turned back to *CLOSED* the instrument records the oscillations under the new compressing pressure thus obtained. By dropping the compressing pressure in this way in steps of 5 or 10 mm Hg determination can readily be made of the arterial pressures by the method of intermittent escapement (see Fig 3).

The remaining positions of the stopcock accomplish what was done by the whole of the cock originally. At *SLOW* the air escapes slowly from the pressure space (through *SP*), and at the same time undue sinking of the tambour head is obviated by an adjustable leak, *ST*. The arrangements are the same as at *FAST* except that here the adjustments (by the screws over *FP* and *FT*) should be such as to permit of a somewhat more rapid fall of the compressing pressure. At *OUT* the tambour space and the pressure space both open freely to the exterior. The rates of an escape from the pressure space

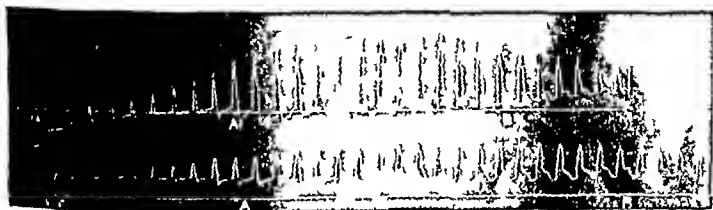


Fig 7.—Two records made in succession from the same subject by the method of continuous escapement the upper with 12 cm of band tubing exposed the lower with 5.5 cm. The marker signalling the sounds writes 3 mm ahead of the pulse marker in the upper and 3 mm ahead in the lower record. "X"

and the rates of entrance of air into the tambour space are determined by adjustment of the screws in the stopcock all of which are now plainly in view and accessible from above (see Fig 5).

In Fig 7 are recorded two determinations made by the method of continuous escapement with the new apparatus. Both tracings were obtained from the same subject, the upper with the sliding sleeve withdrawn so as to expose 12 cm of the hand tubing in the sphygmoscope, the lower with 5.5 cm of the hand tubing in use. The advent of the first Korotkoff (pistol shot) sound and the change of the sounds from those of the third to those of the fourth phase (dulling) were objectively signalled while making these records. The marks, *A*, signalling the advent of sound, fall, as can be seen, 1 to 2 pulses later than the graphic sign (the first change in the form of the pulse). And the marks *B*, signalling the dulling of the sounds, fall precisely at the point where the accelerating decline in pulse amplitude changes to a retarding decline.

A CONVENIENT METHOD OF COLLECTING SMALL AMOUNTS OF SERUM*

BY MAX SHAWEKER, M D , DOVER, OHIO

IT FREQUENTLY occurs in the collection of small amounts of serum, such as complement serum from the guinea pig, that a few red blood cells wash away from the clot and interfere with pipetting off the serum without considerable waste. A method which we use, easily removes this objection and gives the maximum yield of serum from a given amount of blood. The process consists of collecting blood from cardiac puncture, carotid section, venipuncture or other method and placing it in Petri dishes at room temperature for a half hour. About 5 to 10 c c is the optimum amount of blood per standard 100 mm dish. The dish is slanted slightly so that the blood will not completely

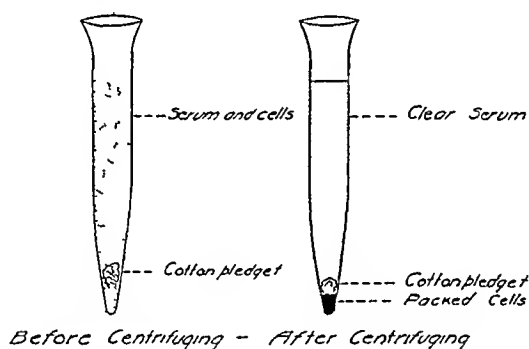


Fig 1

cover the bottom of the dish, leaving a small area in which to collect and pour off the serum. After the clot is well organized it is cut with a needle or knife in small lines radiating to the bare spot in the Petri dish. The serum then gravitates to the bare side of the dish taking perhaps an hour or even overnight, as in guinea pig complement collection. Into a dry 15 c c centrifuge tube is placed a small piece of absorbent cotton. A little shaking may be required to throw it to the bottom of the tube. The serum from the Petri dishes with any reasonable amount of red blood cells which loosen from the clot is now poured into the centrifuge tube. After a brisk centrifuging, the cells will gravitate through the cotton and pack below it. Then the tube can be completely inverted and the serum decanted without disturbing the cells packed under the cotton pledget. Serum so collected is entirely free of turbidity due to accidental admixture of red blood cells and is usually hemoglobin stained.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILRUFFE MD ABSTRACT EDITOR

Pack, G. T. The Pathology of Burns Arch Path and Lab Med, May, 1926, 1, No 5, 767

Various etiologic agents may be responsible for causing burns and scalds dry heat, moist heat of various kinds, the actual flame, heated solid bodies electricity, roentgen rays, radium, sunlight and caustic chemicals

Thermal traumas are best classified in six degrees to denote the various depths of tissue invaded or destroyed. The local tissue changes progress through the various stages of destruction or burning, inflammation and sloughing and finally regeneration and repair. The amount of local tissue destruction varies from simple erythema and the degrees of vesication to an involvement of the entire epidermis, dermis, subcutaneous tissues and even muscle and bone, when there is great intensity or prolonged contact of the heat. Scarring is inevitable when the papillary layer of the skin is destroyed.

The liver, brain, bone marrow, and kidneys of the burned patient may exhibit hyperemia, focal necrosis and parenchymatous degenerative lesions. The suprarenal glands are swollen and deep red owing to hyperemia and ecchymotic areas of hemorrhage among the parenchymal cells. The spleen, the lymph glands and the solitary and agminated lymph nodules of the intestinal tract are the seats of toxic focal necrosis occurring in the centers of the germinal follicles. This necrosis is quickly followed by the rapid proliferation of endothelial leucocytes.

The erythrocytes undergo certain alterations in structure and disturbances of function. Leucopoiesis is stimulated by the burn toxin, so that leucocytosis occurs with an increased percentage of neutrophilic polymorphonuclears. A predisposition to thrombosis exists because of the leucocytic disintegration, the venous stasis and the viscosity of the concentrated blood. A goodly portion of the visceral pathology has been attributed to the presence of minute capillary thrombi. The rapid and continuous loss of fluid from the blood in burned patients quickly induces a marked concentration of the blood. This becomes a factor of the greatest importance in the development of the syndrome characteristic of burns, and a factor of prime significance in the fate of the person concerned. Changes observable in the chemical composition of the blood during burns vary at most only slightly from the normal limits.

The urine is subjected to certain quantitative and qualitative alterations, such as oliguria, albuminuria, albumosuria, and acetoneuria.

The hypothetical burn toxin has its source of origin in the burned tissues from whence it is absorbed and circulates in the blood, being carried by the red blood corpuscles. The exact nature of the burn toxin is as yet unknown, but it is probably closely related to the primary and secondary proteoses or to other products of protein disintegration.

The most common complication of burns and scalds is a secondary pyogenic infection of the burned area. Other complications which occasionally occur are nephritis, septicemia, tetanus, wound hemorrhage, meningitis, apoplexy, pneumonia, amyloid infiltration of the viscera and cicatricial contractural deformities. The inconstant duodenal ulcer of burns is due either to the irritant action of the bile, which owes its injurious ability to its content of the presumptive burn toxin, or to the production of infarction of the duodenal mucosa by septic emboli.

Electric burns occur at the areas of greatest resistance to the current, particularly at the points of entrance and exit. The electric burn is characterized by extensive sloughing of tissue, delayed healing and the absence of vesicles. The type of lesion produced by lightning varies according to whether the mechanical or thermal action of the electricity pre-

Hodgkin's disease composed the majority of cases. Females tended to have the different forms later in life than males.

At all ages except at the age of puberty the number of males exceeded the females, and a relatively large percentage of women had the condition at or near the time of the menopause.

The duration of lymphoblastoma in the females was apt to be longer than in the males. It tended to be longer in men over 35 years old and in women under 25, than in younger males and older females, except when the latter were from 33 to 44 years of age.

Comparisons between the lengths of the course of disease in irradiated and nonirradiated patients do not indicate that such treatment significantly affects the duration of lymphoblastoma. It may do so in selected cases and yet can be blamed, in cases of short duration in the patients that were below the age of 24, for the greater incidence of those in which irradiation was given than those in which it was not.

The chances of lymphoblastoma lasting long were relatively great for those first irradiated when their disease was in an early stage. However, the duration of any such case can be paralleled by one given no special form of therapy.

Surgery probably can influence beneficially the duration of some cases of lymphoblastoma, particularly if it is employed early and thoroughly and is followed by irradiation.

The type of case usually treated early by surgery or by irradiation is apt to be the same as that destined by nature to last a long time.

The average duration of lymphoblastoma in the 401 deceased patients was 2.76 years, yet about 10 per cent of both the irradiated and nonirradiated had the disease for six or more years. A greater percentage of the seventy-six living patients have had lymphoblastoma this long duration of time. Although among them the frequency of surgical and early irradiation treatment is greater than for the deceased group, the treatment by modern methods will explain at the best but partially the increased percentage of cases of long duration.

Irradiation is undoubtedly of great value to patients with lymphoblastoma, it alleviates symptoms, decreases the size of lesions, and improves the patients' efficiency, in spite of the fact that it does not appear to influence importantly the duration of the disease.

Mann, F. C., and Bollman, J. L. Liver Functional Tests. *Arch. Path. and Lab. Med.*, May, 1926, 1, No. 5, 682.

The most important of the tests suggested for measuring hepatic function have been studied in normal animals, animals with an Eck fistula, animals with permanently reduced amounts of hepatic tissue, and animals with the liver completely removed. As a result of these observations, a few positive statements can be made relative to the functional capacity of the liver, a few suggestive signs of hepatic deficiency can be presented, and the tests of hepatic function suggested can be evaluated by means of a standard technique.

The bilirubinemia which follows removal of the greater portion of the liver is but transitory, and the remaining portion of the liver is soon able to excrete all of the bile pigment formed in the animal. Partial removal of the liver has little demonstrable effect on the rate of disappearance of dyes injected into the blood unless the amount of dye injected is excessive, although complete retention of the dye is easily demonstrated in the completely dehepated animal. The carbohydrate metabolism of animals with greatly reduced amounts of hepatic tissue, so far as tests of hepatic function are an indication, is also maintained at an approximately normal level, and only slight differences from normal may be found in the amount of sugar in the blood. The use of glucose or levulose tolerance tests also fails to bring out any marked deviation of these animals from normal. The marked changes in the formation of urea, accumulation of amino acid and excretion of ammonia, which are specific in the dehepated animal, are difficult to demonstrate in the animal with greatly reduced hepatic tissue. The decrease in the destruction of uric acid, however, may be demonstrated in animals with reduced hepatic tissue. Physiologic reactions following the administration of certain toxic substances may indicate that the reduction in the amount of hepatic tissue reduces the animal's tolerance to these substances, but the chemical tests employed do not show any extensive decrease in the rate of conjugation of these substances.

By this procedure the tubercle bacilli are almost always found. They are usually granular and arranged in masses. Sometimes they are isolated and some are found in contact with polymorphonuclears. There may be only a very few but with a good preparation and with a properly lighted microscope they can be easily seen.

When this procedure is used there is no danger of mistaking smegma bacillus for the tubercle bacillus as all the bacteria seen are tubercle bacilli.

By this method positive results may be expected in 88 per cent of cases.

Reiter H. The Cultivation of Pure Cultures of *Spirocheta Pallida*, *Spirocheta Dentium* and *Spirocheta Recurrentis*. Klin Wchn. March 12 1926, v. 444

The best medium for *Sp. pallida* and *Sp. dentium* is a mixture of equal parts of horse serum and normal saline with sterile pieces of rabbit or guinea pig kidney or liver.

The following are useful liquid media.

A Liquid medium for *Spirocheta pallida*

1 Normal rabbit serum is mixed with a 1 per cent normosal solution and pieces of the brain of these animals are inserted. This mixture is kept for twenty four hours at a temperature of 36 C. Its sterility is controlled for twenty four hours at a temperature of 37 C.

The inoculation is performed with capillaries. Sterile paraffin oil or white vaseline is used for the covering. The incubation is made at 37 C.

The optimum of the growth is on the fourth or fifth day. The cultures remain transparent and odorless. The reinoculation is made on the seventh day.

2 Human or cattle liver is kept for forty eight hours at a temperature of 37 C. The secreted fluid is filtered and its sterility is controlled. This autolyate is mixed in equal parts with a 1 per cent normosal a citis (autolyate normosal a citis).

The inoculation, covering and incubation are the same as in 1.

The optimum is on the second day. There is no macroscopic change in the medium and no odor. The reinoculation is made on the fourth day.

B Liquid medium for *Spirocheta dentium*

1 Normal mutton serum is mixed with a 1 per cent normosal solution and pieces of rabbit or guinea pig brain are inserted. Heating and sterility test as in 1 A.

The inoculation, covering and incubation are the same as in 1 A.

The optimum is on the fourth or fifth day.

The smell is typical for *Sp. dentium*; the macroscopic opacity is seen to settle in the form of a greyish white cloud. The reinoculation is made on the seventh day.

2 This is the same procedure, only replacing mutton serum by rabbit serum. The growth is slower, the optimum is on the fifth or sixth day. The smell is typical but there is no sedimentation.

C Liquid medium for *Sp. recurrentis*

The medium is the same as for *Sp. pallida* but the addition of a quantity of fresh sterile guinea pig blood amounting to 10 per cent of the total is necessary.

The inoculations, covering and incubation are the same as in 1 A.

The optimum is on the fifth day. There are no macroscopic changes. The reinoculation is made on the sixth day.

Minot, G. R. and Isaacs, R. Lymphoblastoma (Malignant Lymphoma). Age and Sex Incidence, Duration of Disease and the Effect of Roentgen ray and Radium Irradiation and Surgery. Jour. Am. Med. Assn. April 17 1926, lxxxvi, 1185, April 24 1926, lxxxvi, 1265.

Four hundred and seventy seven cases of all types of lymphoblastoma excluding lymphatic leucemia, have been studied. Four hundred and one of the patients are dead, 238 were treated by roentgen ray or radium irradiations, and 163 were not. Sixty eight per cent of all were males.

The ages at which lymphoblastoma most frequently began were from 20 to 24, when most of the cases were of the Hodgkin type. The next ages when persons were most susceptible were from 35 to 39. At this time in life other forms of lymphoblastoma than

Hodgkin's disease composed the majority of cases. Females tended to have the different forms later in life than males.

At all ages except at the age of puberty the number of males exceeded the females, and a relatively large percentage of women had the condition at or near the time of the menopause.

The duration of lymphoblastoma in the females was apt to be longer than in the males. It tended to be longer in men over 35 years old and in women under 25, than in younger males and older females, except when the latter were from 33 to 44 years of age.

Comparisons between the lengths of the course of disease in irradiated and nonirradiated patients do not indicate that such treatment significantly affects the duration of lymphoblastoma. It may do so in selected cases and yet can be blamed, in cases of short duration in the patients that were below the age of 24, for the greater incidence of those in which irradiation was given than those in which it was not.

The chances of lymphoblastoma lasting long were relatively great for those first irradiated when their disease was in an early stage. However, the duration of any such case can be paralleled by one given no special form of therapy.

Surgery probably can influence beneficially the duration of some cases of lymphoblastoma, particularly if it is employed early and thoroughly and is followed by irradiation.

The type of case usually treated early by surgery or by irradiation is apt to be the same as that destined by nature to last a long time.

The average duration of lymphoblastoma in the 401 deceased patients was 2.76 years, yet about 10 per cent of both the irradiated and nonirradiated had the disease for six or more years. A greater percentage of the seventy-six living patients have had lymphoblastoma this long duration of time. Although among them the frequency of surgical and early irradiation treatment is greater than for the deceased group, the treatment by modern methods will explain at the best but partially the increased percentage of cases of long duration.

Irradiation is undoubtedly of great value to patients with lymphoblastoma, it alleviates symptoms, decreases the size of lesions, and improves the patients' efficiency, in spite of the fact that it does not appear to influence importantly the duration of the disease.

Mann, F. C., and Bollman, J. L. Liver Functional Tests. *Arch. Path. and Lab. Med.*, May, 1926, 1, No. 5, 682.

The most important of the tests suggested for measuring hepatic function have been studied in normal animals, animals with an Eck fistula, animals with permanently reduced amounts of hepatic tissue, and animals with the liver completely removed. As a result of these observations, a few positive statements can be made relative to the functional capacity of the liver, a few suggestive signs of hepatic deficiency can be presented, and the tests of hepatic function suggested can be evaluated by means of a standard technique.

The bilirubinemia which follows removal of the greater portion of the liver is but transitory, and the remaining portion of the liver is soon able to excrete all of the bile pigment formed in the animal. Partial removal of the liver has little demonstrable effect on the rate of disappearance of dyes injected into the blood unless the amount of dye injected is excessive, although complete retention of the dye is easily demonstrated in the completely dehepated animal. The carbohydrate metabolism of animals with greatly reduced amounts of hepatic tissue, so far as tests of hepatic function are an indication, is also maintained at an approximately normal level, and only slight differences from normal may be found in the amount of sugar in the blood. The use of glucose or levulose tolerance tests also fails to bring out any marked deviation of these animals from normal. The marked changes in the formation of urea, accumulation of amino acid and excretion of ammonia, which are specific in the dehepated animal, are difficult to demonstrate in the animal with greatly reduced hepatic tissue. The decrease in the destruction of uric acid, however, may be demonstrated in animals with reduced hepatic tissue. Physiologic reactions following the administration of certain toxic substances may indicate that the reduction in the amount of hepatic tissue reduces the animal's tolerance to these substances, but the chemical tests employed do not show any extensive decrease in the rate of conjugation of these substances.

In summarizing the results of the experiments, as they would appear to apply to the clinical tests of hepatic function, the following can be suggested. There appears to be no physiologic basis for many of the tests employed to measure the functional capacity of the liver. So far as carefully controlled experimental data may apply to such problems, most of the tests should be discarded. Some of these tests which could not be proved experimentally to have any value in a known and controlled condition of hepatic deficiency may be of value clinically, either because they are an index of disease, not necessarily wholly hepatic, or because spontaneous disease of the liver may affect the function differently from experimental procedures, or because man may be somewhat different from the dog. However, the value of such tests should be accepted only with data obtained in cases in which the hepatic disease is proved either by a definite clinical diagnosis or anatomically at operation or necropsy.

The tests which would appear from these experimental data to have value in relation to estimation of the function of the liver are as follows. The van den Bergh tests for bilirubin in the blood should be of value as a measure of a condition in which the liver is either directly or indirectly, but not necessarily predominantly, affected. The physiologic basis for the use of the dyes which have been employed as tests of hepatic function is the fact that they appear to be excreted mainly by the liver. Experimentally, the hepatic function could not be sufficiently diminished to show a definite relation of hepatic insufficiency to retention of dye. Since, however, there is a definite retention of the dye in certain cases in man, this would appear to be one of those tests whose value can be determined only by its careful and controlled use clinically as has been done by Rowntree and his associates. There is a definite experimental basis for the elaboration of a test of glycogen mobilization which might bear the same relation to the activity of the liver in relation to carbohydrate metabolism as the quantitative bilirubin test does to pigment metabolism.

The best test of functional deficiency of the liver in the dog which has been found is based on the facts that destruction of uric acid depends on the liver, that this is the most easily injured of the known functions of the liver, and that the amount of uric acid excreted in the urine appears to bear a definite relation to hepatic damage. This test is easily performed by determining the amount of uric acid eliminated in the urine during a standard period of time following the ingestion of a standard meal with high content of purin. Whether or not such a test will be of value in man remains to be proved.

Plant, A. Bilharzia Infection in an Apparently Normal Appendix. Arch Path and Lab Med., May, 1926, 1, No 5, 712

Report, illustrated with eleven microphotographs of a case in which ova of *Schistosoma hematobium* were found in sections of an apparently normal appendix removed in the course of an operation for retroversion. Ova were later found in the feces and in sections from the cervix.

Weidman, F. D. and Sunderman, F. W. Hypercholesterolemia. The Normal Blood Cholesterol Figures for Man and the Lower Animals. Arch Dermat and Syph., November 1925, xii, 679

A review of the findings of various workers using a variety of methods: gravimetric, colorimetric, and spectrometric, and of the authors' results with the Myers-Wardell technique modified by Karr and Oser.

The normal cholesterol values were determined for four men, three monkeys, four dogs, four cats, four rabbits and three guinea pigs, each being tested on four or five occasions.

The average normal values obtained were:

Man	140-160 mg per cent
Monkey	121-136 mg per cent
Dog	111-118 mg per cent
Cat	91-100 mg per cent.
Guinea pig	81- 87 mg per cent.
Rabbit	64- 80 mg per cent.

Hodgkin's disease composed the majority of cases. Females tended to have the different forms later in life than males.

At all ages except at the age of puberty the number of males exceeded the females, and a relatively large percentage of women had the condition at or near the time of the menopause.

The duration of lymphoblastoma in the females was apt to be longer than in the males. It tended to be longer in men over 35 years old and in women under 25, than in younger males and older females, except when the latter were from 33 to 44 years of age.

Comparisons between the lengths of the course of disease in irradiated and nonirradiated patients do not indicate that such treatment significantly affects the duration of lymphoblastoma. It may do so in selected cases and yet can be blamed, in cases of short duration in the patients that were below the age of 24, for the greater incidence of those in which irradiation was given than those in which it was not.

The chances of lymphoblastoma lasting long were relatively great for those first irradiated when their disease was in an early stage. However, the duration of any such case can be paralleled by one given no special form of therapy.

Surgery probably can influence beneficially the duration of some cases of lymphoblastoma, particularly if it is employed early and thoroughly and is followed by irradiation.

The type of case usually treated early by surgery or by irradiation is apt to be the same as that destined by nature to last a long time.

The average duration of lymphoblastoma in the 401 deceased patients was 276 years, yet about 10 per cent of both the irradiated and nonirradiated had the disease for six or more years. A greater percentage of the seventy-six living patients have had lymphoblastoma this long duration of time. Although among them the frequency of surgical and early irradiation treatment is greater than for the deceased group, the treatment by modern methods will explain at the best but partially the increased percentage of cases of long duration.

Irradiation is undoubtedly of great value to patients with lymphoblastoma, it alleviates symptoms, decreases the size of lesions, and improves the patients' efficiency, in spite of the fact that it does not appear to influence importantly the duration of the disease.

Mann, F. C., and Bollman, J. L. Liver Functional Tests. Arch. Path. and Lab. Med., May, 1926, 1, No. 5, 682.

The most important of the tests suggested for measuring hepatic function have been studied in normal animals, animals with an Eck fistula, animals with permanently reduced amounts of hepatic tissue, and animals with the liver completely removed. As a result of these observations, a few positive statements can be made relative to the functional capacity of the liver, a few suggestive signs of hepatic deficiency can be presented, and the tests of hepatic function suggested can be evaluated by means of a standard technique.

The bilirubinemia which follows removal of the greater portion of the liver is but transitory, and the remaining portion of the liver is soon able to excrete all of the bile pigment formed in the animal. Partial removal of the liver has little demonstrable effect on the rate of disappearance of dyes injected into the blood unless the amount of dye injected is excessive, although complete retention of the dye is easily demonstrated in the completely dehepated animal. The carbohydrate metabolism of animals with greatly reduced amounts of hepatic tissue, so far as tests of hepatic function are an indication, is also maintained at an approximately normal level, and only slight differences from normal may be found in the amount of sugar in the blood. The use of glucose or levulose tolerance tests also fails to bring out any marked deviation of these animals from normal. The marked changes in the formation of urea, accumulation of amino acid and excretion of ammonia, which are specific in the dehepated animal, are difficult to demonstrate in the animal with greatly reduced hepatic tissue. The decrease in the destruction of uric acid, however, may be demonstrated in animals with reduced hepatic tissue. Physiologic reactions following the administration of certain toxic substances may indicate that the reduction in the amount of hepatic tissue reduces the animal's tolerance to these substances, but the chemical tests employed do not show any extensive decrease in the rate of conjugation of these substances.

Figures are also quoted from the literature for various other animals

As far as can be told at present the normal values for man lie between 160 and 180 mg, though influenced by diet and the method employed

In animals there is no apparent relation between the diet and the blood cholesterol

There are so many possibilities of error in current colorimetric methods that each worker should first run normal controls for himself when dealing with animals

Zamorani, V Bilirubin in Feces and Meconium of Nursing Infants *Clin Pediatr*, 1925, *xviii*, 9

The feces of nursing infants contain unaltered bilirubin, the feces of children over one year of age contain only urobilin and urobilinogen

Bile secretion begins in the sixth fetal month and biliverdin appears in the meconium. In the first eight days of life the bilirubin excretion is about 0.13 per cent of the feces, decreasing to 0.01 per cent after six months

Meulengracht, E., and Iverson, P Blood Sugar in Pernicious Anemia *Deutsch Klin Med*, 1925, *xviii*, 1

In a series of 250 examinations a moderate increase in blood sugar was noted in pernicious anemia. There was no apparent relation between blood sugar and the hemoglobin content

During acute stages the blood sugar curve may be raised or lengthened due, not to the anemia, but to the accompanying intoxication

Bueltemann, H., Lange and Huer's Photochemical Test of Serum Applied in Gynecology *Munch med Wchn*, Feb 5, 1926, *lxviii*, 238

To 0.1 cc of fresh serum add 0.2 cc of 0.1 per cent silver nitrate solution

The reaction is read as negative (no sediment, supernatant fluid dark brown), or positive (dark brown sediment and limpid supernatant fluid), the positive reactions being graded II, III, and IV in accordance with the amount of sediment and the clarity of the supernatant fluid

Bueltemann found positive reactions in carcinoma, operable cases giving II and III reactions, inoperable, IV

In pregnancy, as well as recent and uncomplicated abortion, the reaction was consistently negative

After delivery and all during lactation the reaction was positive. In febrile conditions occurring during pregnancy or in any febrile reaction the test was positive

The reaction is held to result from a displacement of the proportional protein globulin content of the serum

Bassler, A A Quantitative Test of Digestive Pancreatic Activity Easily Applied *Clinically* *Arch Int Med*, February, 1925, *xxv*, 162

Technic of test—The reagents are 1. A standard starch P_H 6.7 sodium chlorid solution made up as follows

To solution A, when cool, 50 cc of solution B (plain water acts quite as well), and 25 cc of solution C and distilled water should be added to bring the volume of the mixture to exactly 400 cc. This should be freshly prepared

Solution A In a beaker, 2 gm of the starch to 100 cc of cold distilled water should be mixed thoroughly and then heated. Under constant stirring, this should be brought to boiling and then cooled

Solution B is a standard buffer solution P_H 6.7, 50 cc fifth molar acid potassium phosphate and 21 cc fifth molar sodium hydroxide solution are accurately delivered in a 200 cc volumetric flask, and the contents brought to the exact volume with distilled water

Solution C is a 1 per cent sodium chlorid solution

2. A twenty fifth normal iodine solution, with a dropper cork in the bottle

The reagent solution should be ready so that no time is lost in carrying out the test, because pancreatic solution is stated to lose its diastatic efficiency at room temperature at the rate of about 25 per cent in the first twenty minutes after extraction, and 70 per cent in three hours.

The procedure is as follows:

In each of ten tubes (6 by 0.5 inches, 15.2 by 1.2 cm.) in a rack and numbered from 10 to 1, one should accurately pipette with a 1 cc pipette marked in hundredths, fraction 1 of the duodenal return, water and reagent as shown in the accompanying table.

PROCEDURE OF TEST

Number of tube	10	9	8	7	6	5	4	3	2	1
Duodenal return, cc	0.1	0.11	0.125	0.14	0.17	0.20	0.25	0.33	0.5	1.0
Water, cc	0.9	0.89	0.875	0.86	0.83	0.80	0.75	0.67	0.5	0.0
Reagent cc	4	4	4	4	4	4	4	4	4	4
Units (author's) per 100 cc	0	18	16	14	12	10	8	6	4	2

When set up, shaken and ready, the rack is put in an incubator at 38° C or a water bath for thirty minutes. The rack of tubes is then taken out; the contents of each tube is shaken, a drop of the twenty-fifth normal solution of iodine is added to each tube beginning with Tube 1, twenty minutes time is allowed for the changing of colors to become settled, and the estimation of units is made according to the furthest tube to the left that is achromic. The result is then expressed in units of pancreatic enzyme efficiency. No more than one drop of iodine solution should be added because the more the iodine, the deeper the colors, the less definite the comparison and the less accurate the test.

Ordinarily, the tubes having the largest amounts of duodenal return will be green. This shades down from Tube 1, becoming lighter and fainter to one having a colorless content. The achromic tube represents the amount of return from the duodenum that contains one-fiftieth unit of amylase. Generally the tube to the left of this tube shows a light pink or gray and those more toward Tube 10 shade deeper into reddish purple. At the achromic zone, should the right tube be a sage green and the one to the left of it a very light pink, the achromic point would be between these two tubes. For instance, if Tube 5 were green and Tube 6 pink, the units per hundred cubic centimeters would be eleven. The tube that contains the one-fiftieth unit is divided by fifty which gives the units per cubic centimeter. The calculation used is:

Units of amylase in 1 cc duodenal return = units amylase (pancreatic efficiency) per hundred cubic centimeters duodenal return

For instance, in a test that shows achromic in the sixth tube

$$\begin{array}{r} 12 \\ 50 \overline{) 600} \\ 50 \\ \hline 100 \\ 100 \\ \hline \end{array}$$

$$0.12 \times 100 = \text{pancreatic efficiency of 12 units (author's)}$$

It will be noted that the test is so arranged that the result in units is double the number of the tube in which the reaction occurs; thus the sixth tube equals twelve units.

The test, as described takes care of most instances of low pancreatic activity, and meets clinical requirements in a routine way. Should no achromic point be present in the first tube, a greater amount of duodenal return may be used and, according to the amount to arrive at the achromic point, a calculation may be made.

The test is so arranged that the extreme normal ranges occur between the fourth and the seventh tubes (from eight to fourteen units), giving sufficient tubes for errors of the gland on each side of these. The average units in normal persons are between ten and twelve, and, as experience multiplies, we find six units is suspiciously low (slight hypopancreorrhea). Up to the present, in even late carcinoma, marked fibrosis, or acute and sup

purative pancreatitis, the test, as outlined, answered for the low unit readings. In very active secretion, the dilution may be increased and calculated accordingly. The various contents should be added to the tubes, beginning with Tube 10 on the left and working to Tube 1 on the right. This rule should be carried out to the end of the test. This preserves the proper intervals of time in the various additions. Usually, not more than ten minutes are necessary to prepare the tubes in the rack, and this need not be figured off the incubator time (thirty minutes).

Admixture Factor to Foods—It sometimes is desirable to learn what the admixture factor to foods is in the duodenum as a part of the test. A pancreatic juice may contain a greater or lesser amount of enzyme index than normal, it may be hyperfluid or diminished, and stenosis of the duct or at the ampulla may exist. In the test outlined in the above, a juice may very occasionally show a low enzyme index in a unit of return, because the return is watery compared to normal, or it may be too high because of concentration. In abnormal findings of the test described, it is well at times to find out what the admixture factor is, and, while the procedure about to be described has an element of error, it is still accurate enough for clinical purposes. For this test, when the tip of the tube is in the duodenum, the patient drinks the following mixture through a glass tube and lies on the right side. Two hundred c.c. of a 5 per cent starch solution is made up in the usual way, when cooling, 18 gm. of sublimed sulphur is mixed with it in a shake flask (Erlenmeyer), some large faceted beads being used to help in making the mixture uniform. This mixture will hold the sulphur in uniform suspension up to the end of the test. Having quite a flat taste, the mixture may be flavored with a few grains of saccharin and vanillin crystals.

Two Hopkins vaccine tubes are used in the test. Tube A contains part of the original starch mixture and is used as a control. Tube B contains the return from the duodenum. Each tube is filled accurately to the 5 c.c. mark and centrifugated for about two minutes at high speed. A wire with a slightly bobbed end is run down through the sulphur crystals to rearrange them, and free any bubbles or clots of starch that may have gotten in the small end. They are then centrifugated again for a minute to two, the crystals rearranged as before, and, finally, centrifugated for two minutes more. The difference between the sulphur level in the two tubes gives the admixture percentage.

Example Tube A, 51 spaces, Tube B, 25 spaces

$$\begin{array}{r} 49 \\ 51 \overline{) 2500} \\ \underline{204} \\ 460 \\ \underline{459} \end{array}$$

100 per cent - 49 per cent = 51 per cent admixture of duodenal contents

It must be remembered here that water and starch do not stimulate bile flow, and such flow that may be in a return is trivial. Often, when conducting this test and bile is flowing into the duodenum, as promptly as the starch solution is given by mouth, it is checked. It is better to conduct the admixture test on a subsequent day to the one for enzyme. Obviously, with an enzyme result, and a higher or lower than normal admixture result, the unit reading of enzymatic power would have to be expressed in terms of admixture, almost all of which is pancreatic juice. The range of normal for admixture is from 40 to 80 per cent, with an average normal of 58 per cent in several hundred cases. The lengths of time for the starch to appear in the bottle is from five to fourteen minutes, with an average of six minutes, and the time for sufficient return for the tubes runs from three to twenty five minutes, with an average of fourteen minutes. This test may be employed to diagnose stasis or obstruction in the duct of Wirsung or at the ampulla (diminished quantity of pancreatic juice with normal enzyme activity). This test is only approximately accurate.

Stasis and Obstruction—The patient is prepared as in the foregoing tests with the tip of the tube in the duodenum. Then about 100 c.c. of olive oil is drunk, and the patient lies on the right side. In a few minutes, a return is obtained, and 10 c.c. of this is centrifugated in a graduated centrifuge tube. The percentage of duodenal secretion in this test, almost all of which is bile, in the extreme ranges met with were 59 to 99 per cent, 84 per

cent being the average. The lower the percentage of admixture the surer we may be of the existence of stasis or obstruction in the bile passages. The bile and oil return appears from two to twenty five minutes (average nine minutes), and a sufficient amount for the test in from seven to thirty minutes (average fourteen minutes).

The difference between the admixture result in the starch test for pancreatic juice and the oil test for bile represents bile flow, which in the normal is approximately 35 per cent higher in the second, thus 35 per cent runs true in the low admixtures as well as in the high. Anything below 35 per cent means either excess pancreatic secretion, too low bile secretion, or delivery (biliary stasis). This test is only approximately accurate.

Hardesty W. L. Flocculation in Tuberculosis. Minnesota Med., March, 1926, 119.

Into four test tubes place 0.4, 0.3, 0.2 and 0.1 c.c. of serum which must be clear, not colored by hemoglobin, unheated, and not over one day old.

To 41 c.c. of 2 per cent saline add 10 c.c. of 95 per cent alcohol and to each of the four tubes add 3.3 c.c. of this mixture.

Shake the tubes, heat in a water bath at 60° C. for 30 minutes, let stand for 4 hours and read the degree of flocculation.

This is the technique described by Baum (Baum, J. Am. Rev. Tub., 1924, x, No. 4, 449).

From a study of 300 cases including known tuberculosis, other diseases and normal controls, Hardesty concludes that:

- 1 The reaction is nonspecific.
- 2 In general, tuberculous serum gives reactions the intensity of which is roughly proportioned to the severity of the process.
- 3 Normal serum and serum in diseases other than tuberculosis give negative or only weakly positive reactions.
- 4 The greatest value of the reaction is as an aid in the differentiation of active tuberculosis from other clinically similar conditions.

Cohen M. B., Applebaum, H. S. and Hainsworth E. L. The Intracutaneous Salt Solution Test, Its Use as a Test of the Efficiency of the Circulation in the Extremities. Jour. Am. Med. Assn., May 29, 1926, lxxxvi, 1677.

When 0.2 c.c. of an 0.85 per cent salt solution is injected into the skin of a normal individual sixty minutes or more is required for its complete absorption. When edema is present the disappearance time is decreased in direct ratio to the degree of edema. Disappearances in extreme cases being so rapid as to prevent the production of a wheal. The observations apply to all edema, regardless of its underlying cause, whether cardiac or renal.

This test, devised by McClure and Aldrich (Jour. Am. Med. Assn., May 3, 1924, lxxxii, 1425) is explained as due to the increased affinity of the tissues for water in edema leading to its rapid absorption from the most available source, in this instance the wheal produced by the injection.

During disease processes edema occurs whenever the water holding power of tissue colloids is increased and such increase can be caused by localized acidosis resulting from the accumulation in the tissues of acids, amines, or similar substances.

Localized acidosis can be produced by obstruction of the venous return and patients with varicose veins in whom the degree and duration of stasis can be regulated by posture afforded the authors an opportunity to study the effect of stasis on the salt solution disappearance time.

The following conclusions were formulated as a result of the study:

- 1 The intradermal salt solution test is a rapid and accurate measure of the tissue affinity of water.
- 2 The normal disappearance time is sixty minutes or more.
- 3 In all conditions associated with localized anoxemia the disappearance time is decreased.

BOOK REVIEWS

(Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building, Richmond, Va.)

*The Chemical Analysis of Foods**

THE chemistry and chemical analysis of food is a subject which, within recent years, has undergone an extensive revision and expansion

In this volume the elements of food analysis are presented in a very practical way and particularly for those who have not had special training or extensive experience in this field. The methods given are clearly described and have been well tried.

The scope of the volume is indicated by the following summary of its sections:

Sugars and sugar products (46 pages), starches and cereals (41 pages), baking powder and egg substitutes (11 pages), fruits and vegetables (25 pages), tea, coffee, and chocolate (26 pages), mustard, pepper, and spices (26 pages), wines, spirits, beer and vinegar (34 pages), flesh foods, meat extractives, and gelatin (18 pages), milk, and milk products (40 pages), butter, margarine, and cheese (22 pages), lards and oils (19 pages).

The book deserves a cordial reception from those to whom it is addressed.

A Manual of the Parasitic Protozoa of Man†

EPIDEMIOLOGY, preventive medicine, and even the practice of medicine in general have been very profoundly influenced by the march of progress.

Diseases hitherto confined to certain geographical localities are now to be encountered in far flung places, carried there by modern means of transportation, others, hitherto considered peculiar to certain races, have been encountered in strange places as an aftermath of the unparalleled mixture of peoples during the World War. Especially is this the case in diseases due to protozoa, parasites responsible for some of the most important and serious diseases of man.

Colonel Craig's manual is not primarily a zoological treatise but is intended for the information and use of health officers and practitioners.

In it are presented in a clear, readable, and authoritative manner all the facts of real importance known about the parasites described.

The work is very complete and amply illustrated. Each organism is considered under the following headings: Synonyms, History and Nomenclature, Morphology (living and stained), Resistance, Habitat, Species Found in Lower Animals, Cultivation, Life History, Method of Reproduction, Geographical Distribution, Incidence of Infection, Method of Transmission, Experimental Infection, Relation to Disease, Pathology, Prophylaxis, and Diagnosis.

Under diagnosis all the laboratory methods of value are fully described and a technical appendix contains the most useful cultural and staining methods.

The book forms the most complete, informative, and satisfactory reference on protozoa which has yet been produced for the use of the practitioner, health officer, and laboratory worker, and it seems destined to become a standard in this field. It is attractively printed and deserves a wide circulation.

*The Chemical Analysis of Foods. By H. E. Cox, Public Analyst for The Metropolitan Borough of Hampstead. Cloth. Pp 323. 38 illustrations. Price \$5.00. P. Blakiston's Son and Co. Philadelphia.

†A Manual of The Parasitic Protozoa of Man. By Chas. F. Craig, Lieutenant Colonel Medical Corps U.S.A. Cloth. Pp 569. 95 illustrations. Price \$5.00. J. B. Lippincott Co. Philadelphia and London.

*Clinical Laboratory Methods*⁶

THE second edition of this useful manual will be welcomed by physicians and laboratory workers.

The revision has been extensive and thorough. The chapter on blood has been extended and subdivided into five chapters corresponding to the method of approach in the clinical laboratory.

The section concerning the Wassermann reaction has been rewritten and greatly extended. Kolmer's qualitative and quantitative methods have been included, under the latter, however, the older and more cumbersome serum quantities are given rather than the later quantities incorporated in the method shortly after its publication and which greatly facilitate its performance.

New methods have also been added to the section on blood chemistry and, in fact, useful additions will be encountered throughout the entire book.

Particular care has been taken to make the index comprehensive and to include numerous cross references in both the index and the text.

The illustrations are good, the text well and clearly written, and the book in general in keeping with the standard of the publishers.

It will be found a useful and valuable addition to the library of any laboratory or physician.

Insanity and Law[†]

AS STATED in the preface of this book, there is a marked divergence in the point of view of the physician and the law leading to markedly conflicting opinions in relation to mental health, the physician being concerned primarily with the welfare of the individual, the law paying more attention to the effects of individual inadequacy on the community.

Much of the conflict thus produced depends not upon any fundamental conflict between medicine and law but upon an insufficiency of mutual understanding towards the better development of which this book is directed.

In this book an endeavor is made to state in simple language psychiatric facts which may be regarded as established, to present the legal aspects of mental disorder, and to outline the situation that now obtains both from the medical and legal point of view 'with the hope of bringing about a better understanding of the actual basis from which must start any practical effort to improve the relations between insanity and law. The requirements for this improvement are two fold: the physician needs a better knowledge of legal practice and ideals, and the lawyer needs fuller information about mental diseases' needs which this book attempts to supply.

For this task the authors are qualified by extensive classroom, courtroom and practical experience.

Part I, comprising 198 pages, is devoted to a consideration of mental disorders. A description is first given of certain types of reaction which may be encountered in many different diseases (delirium, hallucinosis, confusional states, dementia, etc.), followed by some account of the principal types of mental disease.

The remainder of the book—Part II—is devoted to the legal aspects of insanity and discusses the legal definition of insanity, the determination of insanity, guardianship of insanity and contracts, insanity and marriage, insanity in relation to tort, insanity and criminal responsibility, insanity and testamentary incapacity, miscellaneous legal aspects of insanity, the physician in court, and some suggestions for reforms in procedure.

A Manual of Clinical Laboratory Methods. By Clyde L. Cummer. Associate Professor of Pathology, Western Reserve University. Second Edition. Cloth. Pp. 547. 169 engravings and 12 plates. Price \$6.50. Lea and Febiger, Philadelphia.

Insanity and Law. A Treatise on Forensic Psychiatry. By H. D. Singer, M.D., Professor of Psychiatry, University of Illinois, and W. O. Krohn, M.D., formerly Head of the Department of Psychology, University of Illinois. Leatherette, \$6.00. P. Blakiston's Son and Co., Philadelphia. 437 pages.

A glossary of terms is appended for the elucidation of the various technical—especially legal—terms necessarily employed

The style is pleasing and as clear and readable as the complexities of the subject permit. A rather extensive experience in the courtroom and a consequent familiarity with—as they appear to the uninitiated—the ponderous pomposities of legal phraseology, lend, at times, a legal flavor to the presentation which seems to necessitate a reference to all judges as “learned” and all Courts as capitalized and partially deified beings of almost incredible wisdom albeit the irreverent might at times find some justification for picturing them as draped, not with the “majesty of the law,” but with the absurdities of unending, tortuous, and obfuscating verbiage.

“The legal aspects of the relations of insanity to the various phases of business and social life have been set forth by quoting and discussing the laws, methods of procedure, and the important decisions of state and federal courts, because familiarity with these decisions will not only enable the physicians better to advise and assist the courts, but will also give a better understanding of the reasons that underlie the various technicalities.”

This is a most interesting and readable section and well repays perusal.

There is much discussion of crime and the criminal these days and even the “learned Bar” has taken cognizance of what the untutored lay mind has long suspected that the legal procedure of the present day is a ponderous structure some of the supports of which are so archaic as to give a somewhat Pisa like tendency to lean more toward the safe guarding of the criminal than toward the protection of the community.

It is somewhat difficult for the unlegal mind—if it may be so termed—to avoid the impression that more attention is paid to what was said by the learned Court in the case of *Woofenpoof vs the people* in 1721, than to whether or not the defendant actually robbed the bank or murdered his aunt, that too many verdicts are nullified on the tenuous grounds of formal technicalities, misplaced commas and similar absurdities, or to understand why it often appears that legal defense procedures are apparently synonymous with attempts to evade the law and its provisions by dexterous and sinuous counter procedures if not by more questionable means.

The perusal of this book will be of value to both the physician and the lawyer. It will not make of any physician a psychiatric expert but it will inform him as to the necessary array and extent of information required by such a position, warn him against rash ventures into such fields, and render him better able to avoid embarrassing situations if forced by circumstances to take the stand either as an ordinary or expert witness.

It will not make of the lawyer a psychiatrist, but furnishes in compact form a mine of information and if honestly read and thoroughly considered should emphasize the necessity of something more than a desire to escape punishment as a reasonable ground for a claim of insanity.

The hypothetical question is discussed at length and its inherent possibilities for misleading the jury noted and the opportunity it affords for legal trickery touched upon, though with all due courtesy to the legal profession and its somewhat tender sensibilities. The extreme latitude allowed in the cross examination of witnesses—which not infrequently resembles bullying—is mentioned in non committal fashion.

A more expanded consideration of needed reforms in procedure seems possible—the experience of the authors could well be brought to bear upon this point. The Bar has it now under consideration and in view of the apparently constitutional inability of the legal mind to divest itself of cumbersome and ponderous technicalities, the medical expert could surely contribute something, providing he is a clear thinking and qualified *expert* and not a professional witness.

As psychiatrists the authors take the orthodox view of the criminal as one requiring treatment rather than corrective measures tending toward the protection of the community of which he is an individual, though this aspect is but briefly touched upon.

In the last analysis this book is a valuable contribution and though most useful to the lawyer and the medico legal expert, it can be read with profit by both professions at large.

*Medicine An Historical Outline**

HE WOULD be brave, indeed, who would suggest additions to the overcrowded medical curriculum, but to advocate some discussion, however brief, of the history of medicine is a stand for which a strong defense may be made

The modern graduate is forced to cover a vast amount of territory unexplored by, and perhaps even unknown to, his predecessors of a few decades ago and yet it may be regretted that, too often, his knowledge and his reading are apt to be restricted to the technicalities of his profession. It is necessary, of course, and an advantage to assay the difficult task of keeping up with medical literature, it is equally advantageous to broaden the field of one's reading—and seldom can this be done with more interest or greater profit than by excursions into medical history.

In the introduction to the "Arabian Nights" occurs the following "The lives of former generations are a lesson to posterity, that a man may review the remarkable events which have happened to others and be admonished, and may consider the history of peoples of preceding ages and be restrained."

In this book are presented the substance of eight lectures in which an outline of medical history from primitive times to the nineteenth century is presented to the students of the Washington University Medical School.

It is obvious that in this limited space no more than an outline can be presented—and no more is attempted. Medical history is a study in transitions and these transitions are vividly as well as attractively sketched. The volume is one which will stand rereading.

The primary purpose is to arouse interest and to stimulate the appetite.

'Books are for nothing but to inspire,' says Emerson and Goethe tells us that 'the best we derive from history is the enthusiasm it excites in us.'

Dr Seelig's subject is interesting in itself and this interest is intensified by his style and manner of presentation.

'If in any vacant vague time you are in a strait as to choice of reading,' Carlyle once said, 'a very good indication . . . is toward some book you have a great curiosity about.' Dr Seelig's outline is well calculated to stimulate and arouse curiosity about the worthies of olden days of whom he speaks entertainingly and so, indirectly, to lead to further reading—and "reading maketh a full man" just as 'studies pass into and influence manners.'

Francis Bacon tells us that 'some books are to be tasted others to be swallowed and some to be chewed and digested' that is read with diligence and attention.

It is difficult to imagine the physician without interest in the history of his profession. If such there be, Dr Seelig's outline will serve without fail to whet his appetite and introduce him to an interesting absorbing and profitable relaxation for his odd moments and leisure hours.

The volume is well printed though not quite attaining the motto of the publishers—sans tache.

A more attractive paper might also have been used.

An introduction by Dr Garrison and numerous plates of outstanding medical celebrities complete a very interesting well written and readable book.

The Nematode Parasites of Vertebrates†

A VERY comprehensive and copiously illustrated determinative manual which should be a useful addition to the reference library of the pathologist and parasitologist.

Medicine An Historical Outline by M. J. Seelig, M.D. Professor of Clinical Surgery, Washington University School of Medicine. Cloth \$ 5.00. Williams and Wilkins Co. Baltimore, Md. Pp. 707. 27 illustrations.

†*The Nematode Parasites of Vertebrates* W. Yorke, M.D. Professor of Parasitology, University of Liverpool and P. A. Mapstone, M.D. with an Introduction by G. W. Stiles, Professor of Zoology, U. S. Public Health Service. Cloth \$9.00. P. Blakiston's Son and Co. 526 pages, 307 illustrations.

unusual conditions, but we feel that he has failed to include certain diseases which should be discussed and has perhaps gone into unnecessary detail with others. The medical section of the book is the smallest, the surgical the largest. In the former we should like to see some mention of those most common of medical conditions, nephritis, hypertension, arterio sclerosis and cardiac disease. We feel that for the nurse a knowledge of the treatment of nephritis and diabetes is more important than the treatment of gonorrhea in the male.

Likewise in the obstetric and gynecologic sections we should like to see more details of the relationship between pregnancy and acute and chronic disease in the mother.

Aside from this difference of opinion as to what subjects should be included in such a text, the volume appears to fill excellently the function for which it was intended. The description of operations is sufficiently detailed so that the nurse may know the rationale thereof.

We are glad to note that the author recommends preliminary medical treatment in gastric and duodenal ulcer before resorting to surgery. His discussion of the use and indications and counterindications for pituitrin in obstetric practice is excellent. In the orthopedic section we find good illustrated descriptions of reconstructive exercises such as are usually applied by the nurse. A most important chapter is on constipation. Far too many patients nowadays develop the cathartic habit while in the charge of a nurse. The directions for the nursing care of acute conditions such as appendicitis before and after operation is presented in a better manner than usual.

The section on pediatrics covers particularly appropriate subjects for the information of the nurse. We hope that in the next edition more space will be available for a discussion of nutritional disorders in infancy and childhood and newer methods for their treatment and prevention.

Comparative Physiology¹

THE science of comparative anatomy and embryology has perhaps more than any one method of investigation enabled us to understand clearly the process of fetal development.

Much of our present knowledge of human and mammalian physiology is based on studies of comparative physiology. Indeed, we feel secure in the statement that in spite of tremendous recent advances resultant on increasing knowledge of such sciences as biochemistry, much of our future advancement in physiology and functional pathology will still be dependent upon studies in comparative physiology.

Take, for example, the function of the kidney. Does the glomerulus merely serve as a filter allowing the blood crystalloids to pass through, separated from the blood proteins? Is the secretion of the urine through the glomeruli dependent merely upon the balance between the vascular blood pressure and the opposing osmotic pressure? Does the tubular epithelium secrete actively or does it resorb? While these questions have not as yet been conclusively answered much light has been shed upon them by the fact that in other animals in which the anatomic arrangement is different from that of mammals, we may study individual functions. Thus in the frog the glomeruli receive vessels from the aorta while the tubules are supplied with blood vessels from the portal system. One blood supply may be cut off with the other left intact. This has given us the definite information that with the supply to the glomeruli cut off, the kidneys do not secrete urine. Again, certain fishes have such a low blood pressure in the aorta that in studies of the physiology of urinary secretion we may eliminate this factor to a certain extent. It seems unlikely that there is ever a blood pressure in the renal vessels of the fish sufficient to overcome the osmotic pressure of the blood colloids. If this is actually the case, filtration clearly plays no part in the renal function of fishes. True, the last word has not been said in renal function but without comparative physiology, our knowledge would be considerably retarded.

The *magnum opus* on this subject up to 1910 has been Winterstein's *Vergleichende Physiologie*. This is of encyclopedic proportions and should be known to all who are in

¹Comparative Physiology. By Lancelot T. Hogben, M.A. (Cantab), D.Sc. (Lond). Assistant Professor in Zoology, McGill University. Cloth. Pp. 219. The Macmillan Company, 1926.

interested in the subject. The present contribution, while covering the realm of general physiology, aims particularly to bring out the works of importance which have appeared since 1910. The author divides his discussion into four sections. The first to manifestations of vital activity such as muscular contraction, capillary activity, ameboid reactions, color response and secretion, the second to metabolism or the sources of vital energy, including respiration nutrition and circulation of body fluids, the third to coordination, the integration of vital activity, including endocrine coordination, the mechanism of nervous conduction and the analysis of behaviour in animals the fourth to reproduction, the building up of a new animate unit. Here the author discusses comparative knowledge obtained on the fertilization of the egg, inheritance and the physiology of development. Taken as a whole, the volume is quite complete in its outlook.

Aside from its scientific interest to students of zoology or physiology the work is delightful general reading in that it explains curious everyday phenomena such as the change of color of the lizard or toad the mechanism of movement of the ameba respiration in fishes, the manufacture of poison in salivary glands, of strong sulphuric acid in salivary glands, the storage of oxygen in gas bags in certain fishes, such as the "puffer fish" or balloon fish and the secretion of luminous material.

*Kidney Diseases**

A MANUAL for victims of nephritis and hypertension written along lines analogous to those followed by Joslin in his famous manual for diabetics. The basis of treatment outlined by the author is primarily salt restriction, especially in hypertension. At the same time proper emphasis is placed on nitrogenous restrictions and other therapeutic measures. Thus being in essence a manual for the use of patients and physicians, the author does not include any large volume of statistical or experimental evidence substantiating his contention that an error of salt metabolism is a basic factor in the etiology in hypertension, but he rather goes ahead placidly assuming that this has been conclusively demonstrated and outlines treatment on this basis. Dr. Allen is convinced that in his hands radical salt restriction has produced better results than any other single method. In his introduction he writes "If the proposals are valid they will revolutionize the treatment of high blood pressure and effect a corresponding saving of health and life. If they are mistaken, the writer persists in the mistake after six years of careful study and is prepared to bear the consequences."

The author's conception of the etiology of diseases of the kidneys blood vessels and pancreas as a tissue sensitization consequent on a primary injury, usually a severe acute infection is most logical. Even though perfect recovery appears to follow this infection, the kidneys and blood vessels are left as a *locus minoris resistentiae* for all subsequent infections and intoxications. At the same time the damaged organs probably become abnormally susceptible to functional wear and tear. In this way we can plausibly explain the gradual onset and progressiveness in renal vascular disorders months or years after the primary infectious cause has disappeared and we have justification for the prophylactic use of dietary and other restrictive measures.

The author devotes chapters to diet in general to protein restriction, to salt restriction to the various laboratory procedures, and to useful recipes.

In discussing the laboratory examinations including functional studies, Dr. Allen does not attempt to describe all of those tests even that are in rather general use, but much information is to be gained from a reading of those that he does describe.

While the author does not claim that it is essential in these conditions always to weigh the food he provides simple instructions for accurately weighed and controlled diets. His table of the salt content of foods is the most complete we have seen. He also provides general food tables of acid forming and base forming foods.

*Treatment of Kidney Diseases and High Blood Pressure. By Frederick M. Allen M.D. Part I. Practical Manual for Physicians and Patients. Cloth. Pp. 66. The Physiatric Institute. Morristown N. J.

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS, MO, NOVEMBER, 1926

No 2

Editor-in-Chief WARREN T VAUGHAN, M D

Richmond, Va

ASSOCIATE EDITORS

DENNIS E JACKSON M D	CINCINNATI
PAUL G WOOLLEY, M D	LOS ANGELES
J J R MACLEOD, M B	TORONTO
W C MACCARTY, M D	ROCHESTER, MINN
GERALD B WEBB, M D	COLORADO SPRINGS
VICTOR C MYERS, PH D	IOWA CITY
RUSSELL L HADEN, M D	ROSEDALE, KAS
JOHN A KOLMER, M D	PHILADELPHIA
ROBERT A KILDUFFE, M D	ATLANTIC CITY, N J
A PARKER HITCHENS, M D	MANILA, P I
DEAN LEWIS, M D	BALTIMORE
GEORGE R HERRMANN M D	NEW ORLEANS
HAROLD D CAYLOP, M D	ROCHESTER, MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1926 by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis Mo as Second-Class Matter

EDITORIALS

The Functional Pathology of Nephritis

NEARLY a century ago Richard Bright published his description of a group of cases illustrative of that condition which carries his name. He recognized the occurrence simultaneously of abnormal constituents such as albumin in the urine, and clearly abnormal kidneys at necropsy. He found that the pathologic changes in these organs was not always the same and was unable from study of the urine during life to prognosticate clearly the distribution of structural alterations. Ninety nine years have elapsed and while we know far more concerning both the physiology and the pathology of the kidney we must still confess that even today we usually cannot conclusively localize the renal damage even after having had an opportunity to apply all of the present methods of analytic and blood chemical studies.

In the days when the structural pathologist held dominion, the problem appeared solved and the classification of the chronic nephritides was clearly divided into groups such as parenchymatous, interstitial and glomeruloneph

ritis Weigert then proceeded to demonstrate that chronic parenchymatous lesions are nearly always associated with interstitial growths and interpreted the connective tissue proliferation of interstitial nephritis as an attempt to fill in the defect caused by the destruction of parenchyma. He considered this essentially a replacement fibrosis. Interstitial nephritis then went into the discard.

The classification seemed then to be entirely unambiguous, the large white kidney being associated chiefly with parenchymatous nephritis, the secondary contracted kidney being the end stage of a chronic glomerulo-nephritis and the primary contracted or red granular kidney with its fibrous tissue, enclosing atrophic tubules and obliterated glomeruli and alternating with active parenchyma being the terminal picture of a renal arteriosclerosis.

But the unexpected surprises at necropsy still came too frequently. From a study of the specific gravity of the urine and the relative proportions of albumin and casts and other constituents, anatomic diagnoses were made which were not borne out after death.

Today studies of renal function enable us to ascertain with reasonable certainty the degree of parenchymatous destruction but we are not yet in a position to designate the exact location of the lesion in the individual case. The more recent method of approach to such a localization has been through a minute study of the physiology and pathologic physiology of the organ rather than along anatomic lines. This has carried us quite far but the situation at present is rather anomalous since in certain types of nephritis the "functional" localization of damage is distinctly different from what would appear to have been proved on anatomic grounds. As yet no completely satisfactory reconciliation of this divergence has been reached. Mayrs in discussing parenchymatous nephritis writes as follows: "Hydremic nephritis may be regarded as a disease mainly involving filtration, the glomeruli having become permeable to protein while the function of the tubule cells is less affected. This view seems inconsistent with the microscopic changes found in the kidney. The glomeruli may appear little altered while the tubule epithelium shows cloudy swelling, fatty degeneration and often more serious impairment."

Bowman (1842) postulated the secretion of water through the glomeruli which passing down the tubules washed away the minor excretory products as they were secreted by the tubular epithelium. Ludwig, two years later advanced a theory of the glomerular secretion of a protein-free blood filtrate its crystalline constituents in the same concentration as in the latter with the subsequent reabsorption of water and some of the solid constituents through the tubules. Thirty years after Ludwig's publication Heidenheim believed that he had disproved Ludwig's theory by the fact that certain dyes injected into the circulation appeared to be eliminated by the tubules, the fact that hypothetical increase of pressure in the glomerular vessels following partial obstruction of the renal vein did not increase the output of urine and by the argument that the estimated volume of blood flow through the renal vessels per day was insufficient to account for the amount of urea excreted assuming that it was all excreted purely by glomerular filtration—that the amount of filtration must be incredibly large compared with the volume of blood flow.

Heidenheim hypothesized the active secretion of water and chlorides through the glomeruli, to which was added the urea, uric acid, dyes, etc., which were secreted as the urine passed through the tubules.

In 1917 Cushny formulated the so-called modern theory in which he conceived that a protein free filtrate passes through the glomerulus and that this filtrate contains urea, uric acid, sugar, amino acids and chlorides. In the tubules most of the water and chlorides are reabsorbed together with all of the sugar and the amino acids. The urea passes on in the urine. The value of Cushny's theory lies in the fact that it corresponds more nearly than any of the others to our existing knowledge and it serves to explain more satisfactorily the pathologic changes observed. None would say that it is in its entirety the true explanation of the secretion of urine, but it is surely the best we have.

The experiments of Richards, Wern and their collaborators have clarified greatly our understanding of glomerular function. Prior to their work all experiments on the relationship between blood pressure and blood flow through the kidneys and diuresis had at least two variables. They were able to test the validity of the filtration hypothesis by keeping all recognizable conditions constant with the single exception of the pressure of blood flowing through the vessels of the kidney. They found that under conditions which completely prevented alterations in the rate of blood flow through the kidneys any considerable increase in pressure was associated with a parallel increase in urinary output. This simple fact of mechanics obviously served to corroborate the filtration hypothesis.

They have further examined urine collected from the intracapsular space before it has passed into the tubules and find that this fluid contains no proteins, but does contain both sugar and chloride. Sugar is present in the glomerular filtrate when it is absent in urine collected from the bladder. Chloride is a normal constituent of bladder urine but in frogs which had been kept for a time in distilled water chloride disappeared entirely from the bladder urine and yet was still present in the glomerular filtrate. These facts present "incontestable proof of the reality of absorption within the tubule and provide the basis for further argumentative support of the view that the glomerular function is a physical rather than a vital or secretory one." On the other hand, a slightly greater concentration of urea and of chloride was found in glomerular urine than in the blood plasma. However, they believe that this slight increase can be explained on the filtration hypothesis and does not of necessity indicate a true secretion. The fact indicates, however, the necessity for a study of the manner in which capillary forces and vitality of the glomerular membrane may influence the filtration process. In this connection Mayrs points out that chloride increase is characteristic of a number of capillary exudations (aqueous humor, cerebrospinal fluid) and may be explained by the Donnan membrane equilibrium.

By carefully controlled experiments these workers have shown quite conclusively that constriction of the efferent arterioles leading from the glomeruli causes coincident increase in general blood pressure, diminution of volume of blood flow through the kidney, increase in size of the kidney,

with increase in the size of the individual glomeruli, and an increase in urinary output. Increase in blood pressure causes increased secretion of urine. This increase is effected through constriction of the efferent arterioles.

They have shown that the dilator effect of a constituent of the blood is exerted more markedly on the afferent vessels of the glomeruli than on the efferent, due probably to the fact that the blood in the two vessels is really quite a different fluid, being much higher in colloid concentration in the efferent arterioles. This dilator phenomenon would result in an increase in intra glomerular pressure, and a resulting diuresis.

On the other hand the secretion of urine may be lessened by still greater vasoconstriction such that the afferent vessels are also markedly constricted. Another mechanical factor which serves as a brake on the secretion of urine is the elasticity of Bowman's capsule itself preventing undue distention of the capillary tuft.

White and Schmitt, while not accepting without reservation all of the conclusions reached by Wern and Richards have been able to substantiate their observations on the constitution of the protein free plasma filtrate. They further show that sugar and chlorides are both reabsorbed in the proximal convoluted tubules. Instead of the frog they use *Necturus*, an amphibian whose kidney they believe is better adapted to this form of micromanipulation. In this animal they can obtain fluid from any portion of the proximal convoluted tubule, and they find that sugar while consistently present in the intracapsular fluid, has disappeared by the time the urine has reached half way down the proximal convoluted tubule. Their experiments in demonstrating the site of absorption of chlorides are ingenious but will require confirmation.

So much for the physiology of the secretion of urine. The evidence would indicate that a protein free filtrate passes mechanically through the glomerular capsule and that certain of the constituents particularly sugar, chlorid and water are reabsorbed presumably through the agency of cellular activity as they pass through the convoluted tubules. Whether the passage through the glomerulus is a matter of filtration or whether in addition there is some activity of the epithelial cells covering the tuft has not been conclusively decided, but the weight of evidence favors a simple mechanical filtration. Likewise the possibility of coincident excretion through the tubules has not been absolutely refuted.

Mayr attempts to correlate the symptoms of nephritis and to explain them in the light of what is known of the physiology of the kidney. He recognizes filtration and concentration as the two most important phases in the production of urine, and classifies nephritis in so far as possible into diseases affecting these two functions. For this purpose he adopts MacLean's division into "hydremic" and "azotemic" types as being roughly a distinction between filtration and loss of concentrating power. Acute nephritis with accompanying water retention and chronic parenchymatous nephritis with edema, exemplify the hydremic type while chronic glomerular nephritis with retention of the nonprotein nitrogen constituents is classed as azotemic. Frequently, particularly in the advanced stages, the two types are intermingled.

Chronic parenchymatous nephritis is characterized by an excessive albuminuria, edema, slight or considerable reduction in the volume of urine, retention of chlorides, with at the same time efficient excretion of nonthreshold bodies such as urea. In this condition Mavis believes that the major pathologic change is in the glomeruli rather than in the tubules, the disease involving mainly the function of filtration. The glomeruli, while showing little physical alteration, have become permeable to the serum proteins as in orthostatic albuminuria. The fact that microscopically the glomeruli do not appear greatly diseased is according to the author not conclusive, for this is true likewise of capillaries involved in inflammatory changes elsewhere in the body.

He accepts Epstein's suggestion that a fall in the protein osmotic pressure of the blood plasma is the cause of edema in *hydropic* nephritis. He details the various arguments pro and con and gives confirmatory experimental evidence of his own. Formerly it was held that edema was caused by a hydropemia from failure to eliminate water. This would indicate an increased amount of fluid in the blood but recent observers have found no increase in plasma volume indeed sometimes an actual decrease.

The plasma proteins possess a not inconsiderable osmotic pressure. Starling found that normal plasma could hold water against a pressure of about thirty millimeters of mercury. This property of the plasma protein tends to prevent fluid being forced into the tissues by the force of capillary blood pressure. The loss of protein through the urine so lowers its concentration in the plasma that it no longer holds fluid in the vessels. Water then passes out into the tissues. Where the capillary pressure is high, fluid passes out into the tissues and where the pressure is low it is not reabsorbed as it should be because of the reduced protein osmotic pressure.

Mavis has developed a relatively simple instrument for the determination of the blood protein osmotic pressure and finds that in so-called parenchymatous nephritis there is a very decided drop in this pressure. In these cases the pressure was not much more than a quarter of the normal while at the same time the plasma had quantitatively more than half of its normal protein content. This would suggest a change in the character or proportions of the blood protein constituents. He finds that the determination of plasma osmotic pressure is of diagnostic value in determining the cause of edemas.

The reduced volume of urine in this form of nephritis might be due to impaired secretion through the glomerulus. This appears scarcely a tenable hypothesis if at the same time we presuppose a hyperpermeability to proteins. It may be due to diminished glomerular filtration resultant on a lessened blood flow through the inflamed kidney, or, more fluid may be reabsorbed through the tubules. Mavis feels that probably loss of fluid from the blood into the tissues is compensated as far as possible by excessive reabsorption from the renal tubules.

The author does not look upon chloride retention as a factor of great importance in the causation of nephritic edema. There is usually some retention in this condition but no more than occurs in some other diseases without kidney damage and he has found equally high plasma chloride figures in

cardiac edema. Furthermore chloride retention occurs in diseases not associated with edema.

Mavis suggests that the supposed beneficial effects of calcium in lessening nephritic edema may be due to the action of this chemical in diminishing capillary permeability. In this case the diminished capillary permeability in the glomeruli promotes retention of plasma protein. He feels that this is more likely to be the true explanation than the more commonly accepted one that calcium alters the salt balance in the body.

While he attaches little importance to salt restriction and states that there is no evidence that moderate excess of chloride in the blood causes specific symptoms, he does state that the possibility of disturbances in osmotic equilibrium between the cells and fluids of the body appears to justify the exclusion of salt from the diet.

With regard to chronic glomerulitis or isotonic nephritis the author while recognizing that histologically greatest damage appears in the glomeruli localizes the pathologic functional changes mainly in the tubules. He points out that while many of the glomeruli are badly diseased or even destroyed others which nevertheless remain in a relatively good condition are carrying the burden. The presence of slight albuminuria indicates that there is at least a little functional damage to the glomeruli. Thus provided we assume that protein cannot excrete from the tubules.

As the glomerular filtrate passes through the tubules water is absorbed perhaps by osmosis and chlorides are absorbed actively by the tubule cells. These cells are resistant to the absorption of nonthreshold bodies such as urea, uric acid and creatinine. When however they are damaged as in chronic glomerulo-nephritis their impermeability to these substances becomes lessened and, as water passes through them into the blood, the dissolved nitrogenous substances are not resisted as successfully and they pass back with the water and chlorides.

The cells offer a lessened resistance to diffusion through them of nonthreshold bodies. This resistance is to an extent selective in that cells become permeable to uric acid before they do to urea and only in the later stages do they become markedly permeable to creatinine.

With this diffusion through the cells the blood urea and the blood urea and creatinine gradually increase. The greatest hope of keeping the level of the blood reasonably low consists in diuresis such as usually occurs in chronic glomerulo-nephritis. The promotion of diuresis with caffeine or theobromine should theoretically be logical therapeutics since these drugs appear to act chiefly by increasing glomerular filtration. But it is doubtful whether they could alter an output of urine already considerable. Saline diuretics on the other hand act in the tubules as nonthreshold bodies and merely increase the amount of waste material to be eliminated.

Apparently the renal tubules never lose entirely their ability to actively absorb and concentrate chlorides even in the later stages of chronic nephritis. The function however becomes impaired to an extent.

While Mavis' proposed explanation of the pathologic functional processes is interesting and, taken as a whole presents a picture which can easily

be visualized, much remains to be explained and certain paradoxical situations such as the difference between the proposed functional localization and the known microscopic localization must be reconciled

This review also emphasizes the unsatisfactory state of the present nomenclature MacLean's recent division into "azotemic" and "hydremic" types becomes unacceptable if in the latter there is no actual increase in the fluid content of the blood It would be better for the present at least perhaps to adhere to a nondeterminate classification, such as that recommended by Christian, using such terms as chronic nephritis, nephritis with or without hypertension, chronic nephritis with edema, etc Not until we learn more of the *modus operandi* within the diseased organ can we venture to make a thoroughly satisfactory descriptive classification

REFERENCES

- Richards, A N | Nature and Mode of Regulation of Glomerular Filtration, *Am Jour Sc*, December, 1925, clxx, 781
 White, H L, and Schmitt, F O The Site of Reabsorption in the Kidney Tubule of Necturus, *Am Jour Physiol*, April, 1926, lxxvi, 483
 Mayrs, E B The Functional Pathology of Nephritis, *Quart Jour Med*, April, 1926, xix, 273
 See also discussions of nephritis in the *Book Review Section* of this issue, under "Comparative Physiology" and "Kidney Disease"

—W T V

Vol XII

DECEMBER 1926

DEC 21 1926

No. 3
U. S. Department of Agriculture

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., Editor in Chief
Medical Arts Building,
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.B.
University of Toronto Toronto

Clinical Pathology

W. C. MACCARTY, M.D.
Mayo Clinic, Rochester Minn.

Biochemistry

VICTOR C. JYERS, Ph.D.
State University of Iowa Iowa City

Immunology

JOHN A. HOLMER, M.D.
University of Pennsylvania,
Philadelphia

Bacteriology

A. PARKER HITCHINS, M.D.
Bureau of Science, Manila, I. I.

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

Pathology

PAUL G. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD B. WILBE, M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR, M.D.
Mayo Clinic Rochester Minn.

Experimental Medicine

RUSSELL L. HADEN, M.D.
University of Kansas Pasadena

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY 323 25 First Blvd. St. Louis

Entered at the Post Office at St. Louis Mo. as Second Class Matter

LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment. Estimates Cheerfully Furnished Prompt Service

See our advertisements pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA, PA.



KAHN STANDARD ANTIGEN

for

The Kahn Precipitation Test for Syphilis

~

Kahn Standard Antigen, Bio 104
is supplied in 10-cc vials

A complete direction circular
is enclosed in each package

~

LIST OF LABORATORY DIAGNOSTIC
AGENTS WILL BE SENT
ON REQUEST

~

Medical Research and Biological Laboratories

PARKE, DAVIS & COMPANY
DETROIT, MICHIGAN

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO DECEMBER, 1926

No 3

CLINICAL AND EXPERIMENTAL

AN ANALYSIS OF 500 TUMORS OF THE OVARY*

By G L ROSENBERG M D NEW YORK CITY

THE material considered in this paper consists of 500 ovarian tumors encountered in an examination of 2691 specimens in which both ovaries and both fallopian tubes were submitted for diagnosis. On the basis of the present material the tumor incidence may be placed at 18 per cent. The etiology of ovarian tumors, like tumors in general still remains obscure, and it is still as true as when stated by Frankl in 1914, that a genetic classification of ovarian tumors which is the only classification of any value cannot be given with the present state of our knowledge.

Tumors of this organ may be broadly classified on their gross appearance, as primarily cystic with areas of solid tumor or primarily solid with areas of cystic degeneration, and both of these groups may again be subdivided as regards their innocence or malignancy. One may also group them according to the probable source of origin as derived from the epithelial structures of the organ, i.e., the follicular or germinal epithelium, or as derived from the connective tissue or endothelium. In the present series 75.9 per cent were primarily cystic, 49.5 per cent being classified as simple retention, follicle or corpus luteum cysts, 14.2 per cent as cystadenomas, and 12.2 per cent as dermoid cysts and teratomas.

The retention cysts, which are not true ovarian tumors, can be divided into the group of follicle cysts with or without luteal cell formation and true corpus luteum cysts. The microcystic ovary which is not considered as a tumor and is therefore not included in this summary is interpreted by Frankl as a result of chronic congestion of the ovary although work which has been elsewhere reported suggests another explanation.

*From the Achells Laboratory and the Gynecologic Division of the Lenox Hill Hospital New York City
Received for publication May 6 1926

The retention cysts have a central cavity of varying size which contains a fluid varying in color from water-clear to chocolate brown. The cyst wall is formed either of compressed ovarian tissue, or of connective tissue which may or may not present varying phases of degeneration. The corpus luteum cysts represent the dilated and cystic corpus luteum and their diagnosis is based upon the demonstration of granulosa luteum cells in the characteristic

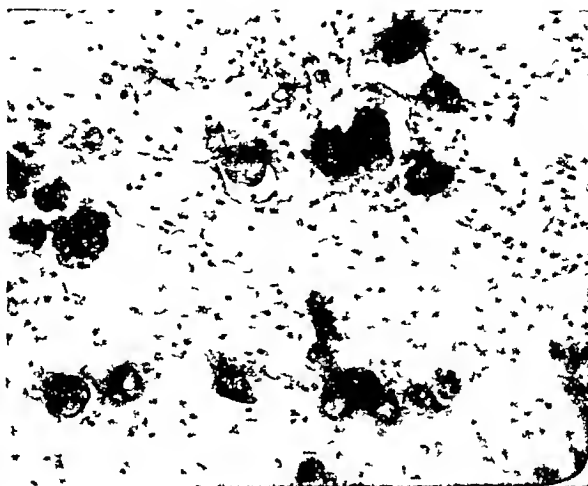


Fig 1—Pacchionian bodies in teratoma x250



Fig 2—Choroid plexus in teratoma. x250

arrangement of the corpus luteum. This cellular layer is rarely present in the larger cysts.

Our material adds nothing new to the present knowledge of these lesions. In the present series one specimen was noteworthy because of its size, the weight being 42 kilos.

Cystadenomas filled with a mucous stringy fluid are the most common of the ovarian tumors, constituting 53.6 per cent of Lippert's 638 ovarian

tumors, while our material showed 71 cases (14.2 per cent). As a rule only one ovary is affected (both ovaries in less than one quarter of the cases, according to Frankl). These tumors possess a connective tissue capsule and a uniformly rounded surface or multiple constrictions according to the dominance in size of different compartments. The size of the individual cysts is variable and in unilocular cystomas one large cyst is seen which may or may not show a variable number of cysts or cystlike spaces in the wall. Some of

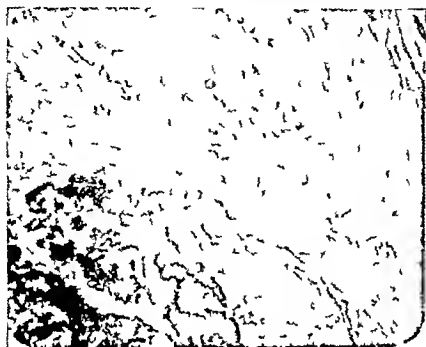


Fig 3—Retinal pigment in teratoma $\times 50$

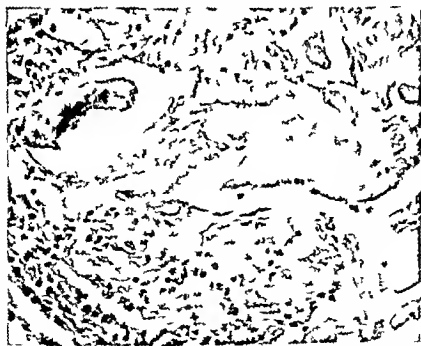


Fig 4—Lipoidal reaction in wall of dermoid cyst $\times 50$

these tumors present an adenomatous structure and closely resemble solid tumors of the ovary. Generally speaking the cystadenomas belong to the group of benign tumors, but in spite of their histologic appearance they may at times assume the properties of a malignant neoplasm. Peritoneal implantations not infrequently manifest themselves after the spontaneous or accidental rupture of a pseudomucinous cystoma. Although it is not a frequent occurrence, these tumors sometimes undergo a histologically demonstrable

carcinomatous degeneration affecting both the primary tumor and peritoneal implantations. Such degeneration is more common in the serous cystadenomas, especially those of the papillary type. The later group contain a serous instead of a mucinous fluid and the inner surface of the cyst spaces is relatively often covered with papillary epithelial growths. Any of the cystadenomas may reach enormous dimensions.

Of greater interest than those previously described is the group of 61 dermoid cysts and teratomas. Kocher claims that the diagnosis of ovarian teratoma or dermoid cysts is rendered possible by the "malleability" of the tumor, meaning by that term that slow prolonged pressure produces a distinct depression which persists for some time. This is due to the putty-like consistency of the cyst contents. Where hair, fluid, and fatty masses are present it is claimed that a fine crepitation may at times also be demonstrated.

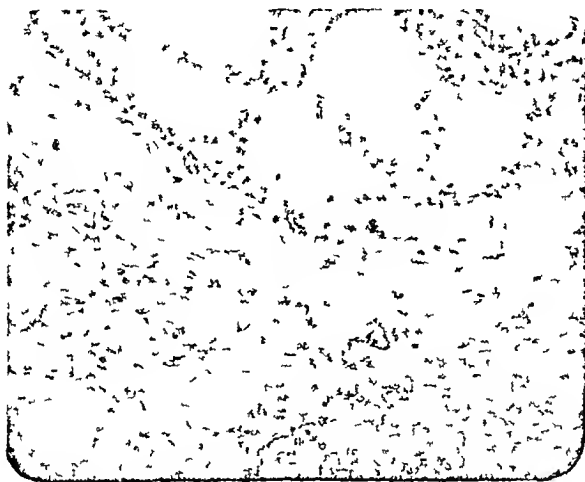


Fig. 5—Thyroid tissue in teratoma $\times 250$

In the present series several points which have been seldom described were encountered. Eighteen of the group were pure dermoid cysts in which the only tissue type demonstrable was the skin and its appendages. In 22 others in which the skin and its appendages formed the major portion of the growth all three fetal layers were demonstrable upon careful study.

In the pure dermoid cyst group, the histologic picture is that of a central cavity filled with sebaceous material and hair, and surrounded by a wall the inner surface of which is a more or less keratinized skin with the usual appendages of sebaceous glands, hair follicles, etc. This epithelial layer is in turn supported either by a connective tissue layer or by a condensation of the ovarian structure. Although seldom commented upon, it was not surprising that 9 instances of lipoidal reaction were encountered. The large amount of lipid present in the sebaceous material is the explanation, and the reaction, which is shown in Fig. 4, is characterized by the formation of foreign body giant cells.

The frequency with which certain tissues were found in teratomas furnished another interesting observation. Brain was encountered 34 times,

and once the frequency of psammoma bodies produced a picture analogous to a psammoma (Fig 1). In one specimen a fully developed choroidal plexus was encountered (Fig 2). Retinal pigment with partial development of the retina was found in 6 instances (Fig 3). Thyroid tissue was demonstrable in 9 specimens (Fig 5), while cartilage, bone, or teeth were found in all of the group.



Fig 6—Choroidal plexus in ovary $\times 50$



Fig 7—Salivary gland in teratoma $\times 50$

Malignant degeneration of the teratomas occurred in 6 specimens (10 per cent). Four of these 6 were classed as carcinomas the cells being of epithelial origin. In all, alveolar formation was a prominent feature and special stains showed no intercellular fibrils of connective tissue. In one of the malignant tumors (Fig 11) a squamous celled epithelioma developed in an area of skin, and metastatic or direct growth extension was demonstrable in the bone and cartilage of the tumor itself.

One instance of carcinosarcoma was shown to be of teratomatous origin by the presence of islands of bone, skin, and muscle

Solid tumors of the ovary are possibly of greater interest than the cystic ones, because they are less frequently encountered. The least common is the adenofibroma, 0.8 per cent of the present material being thus classified. Of the four tumors in this group, three belong to one type and the fourth to another. In the first (Fig 18), the body of the tumor consists of a hyaline or

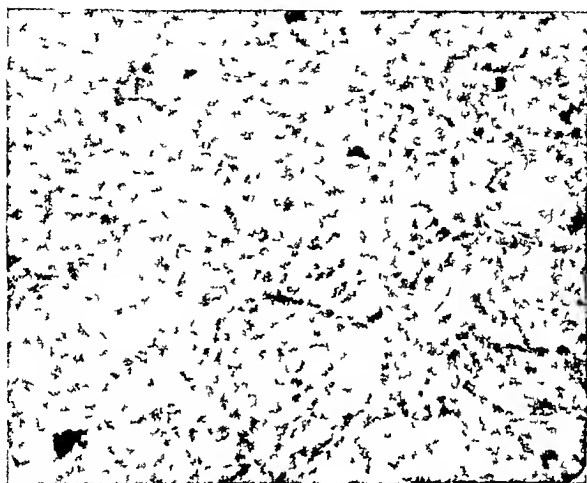


Fig 8—Krukenberg tumor (primary in ovary) $\times 250$



Fig 9—Folliculoma ovarii $\times 250$

slightly cellular stroma arranged with the peculiar manner of infolding observed in intracanalicular tumors of the breast. These papillary-like ingrowths of connective tissue were lined by a single layer of low columnar or cuboidal epithelium which gave no evidence of secretory activity. The single tumor had a more cellular stroma which supported alveoli of varying size, generally ovoid in shape. Most of the cells of these alveoli (Fig 17) showed evidences of secretory activity, and cilia were occasionally demonstrable. Many of

the secretory products had become calcified. This last type is possibly derived from embryonal rests which are not infrequently encountered in the ovary. We have several times observed proliferations of connective tissue about misplaced embryonal ducts, of such degree and so sharply localized that the diagnosis of fibroadenoma of microscopic size was justifiable.

Sixteen specimens of papilloma (3.2 per cent) were found in the tumor group, and as with fibroadenomas there were two types distinguishable. In

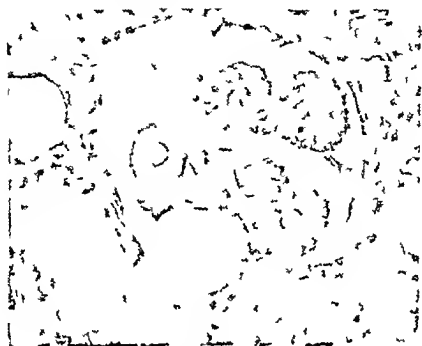


Fig. 10—Folliculoma ovarii $\times 40$



Fig. 11—Squamous cell epithelioma arising in teratoma. $\times 50$

the first type (Fig. 12) the architecture consisted of a dense and rather hyaline connective tissue arranged as papillae and covered by a single layer of low columnar epithelium without evidence of secretory activity. Of this type there were 8 examples. The second type (Fig. 13) had in general a looser connective tissue stroma also arranged in papillary fashion and covered by an epithelial layer which varied from low to high columnar. The characteristic difference between the two types was that the epithelial covering of the

second was of a secreting type, while the epithelium of the first type was nonsecreting

Fibromas of the ovary are solid tumors which usually have a nodular surface. They vary in size from the diameter of a pea up to 40 kilos in weight. The general form of the ovary may have been preserved or it may have merged into the tumor, and usually the tumors are pedunculated. Bilateral tumors are seldom observed. At times the histologic differentiation



Fig 12—Fibroidenoma $\times 250$



Fig 13—Papillary cystadenoma (nonsecreting type) $\times 250$

from sarcoma is difficult, and occasionally the tumors contain varying amounts of smooth muscle. While fibromas are currently supposed to be rather infrequent in the ovary, there were 23 in the present series (46 per cent). In general the tumors consisted of interlacing fasciculi of connective tissue which varied markedly in the degree of cellularity, in many instances being hyaline and in other instances so cellular as to arouse a suspicion of malignancy.

nancy. In some the vascular supply was particularly abundant but in the majority the blood vessels showed a marked degree of sclerosis.

Two possible sources of origin were demonstrable in the series. In one specimen (Fig 19) in which there were multiple fibromas small tumors could be definitely shown to arise from the adventitia of small blood vessels. A similar source of origin has also been shown for fibromas in other portions of the body. In another specimen (Fig 20) an area of struma ovarii in the



Fig 14—Papillary cystadenoma (centering top) $\times 50$



Fig 15—Adenocarcinoma arising from cystadenoma $\times 80$

center of the fibroma suggested that the growth might have been of congenital origin.

Degenerative changes encountered in the fibromas are of more than passing interest. Hyaline degeneration, necrosis attributable to interference with blood supply, and hemorrhage with liquefaction were encountered with fair frequency as is the case with uterine tumors of similar type. Salcom

of metastatic origin. The tumor may start as a malignant growth or arise secondarily from a previously benign one. Most of the secondary carcinomas have the primary growth situated in the gastrointestinal tract. As with carcinomas in other locations extreme youth is not exempt, and tumors of this type have also been observed in hermaphrodites.

The 64 carcinomas of the present series do not include the teratomas in

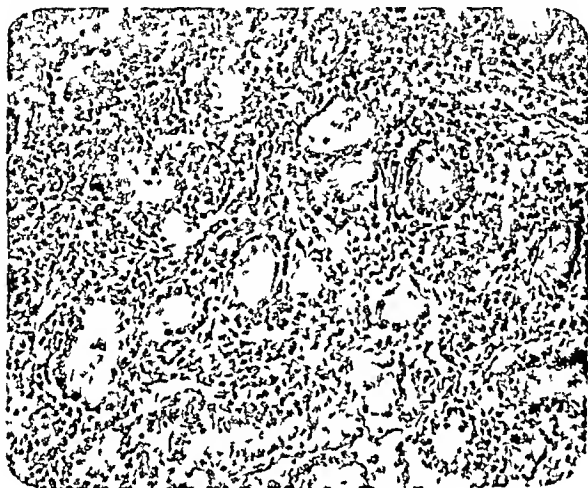


Fig. 20—Stroma ovum $\times 250$

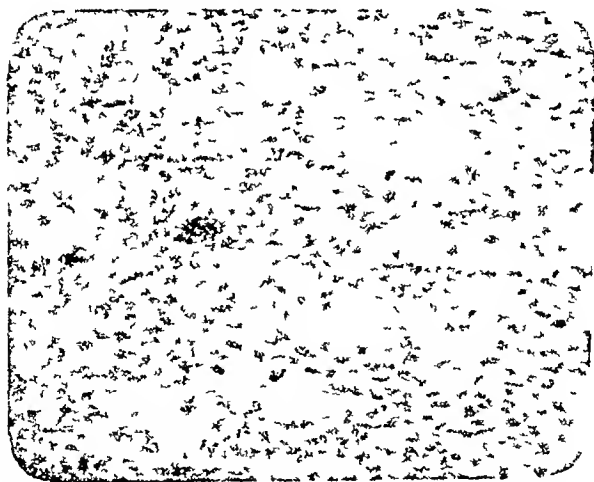


Fig. 21—Degenerative change in fibroma (early phase) $\times 250$

which malignant degeneration occurred. The neoplasms could be about equally divided in two groups. In the one group the tumor consisted of solid masses of cuboidal, polyhedral, or cylindrical cells arranged about more or less delicate septa. Depending upon the plane of section, the architecture differed from a solid sheet of neoplastic cells to the appearance of Fig. 16, where the structure is of the papillary type. The epithelial cells of this type of tumor showed no evidences of secretory activity.

The second group showed a morphology which indicated the origin from cystadenomas. The stroma was of varying degrees of cellularity and arranged so as to support acini of various shapes and sizes. The acini were lined by many layers of columnar epithelium and the lumen of the alveoli as well as the epithelial cells were more or less distended with the retained products of secretion.

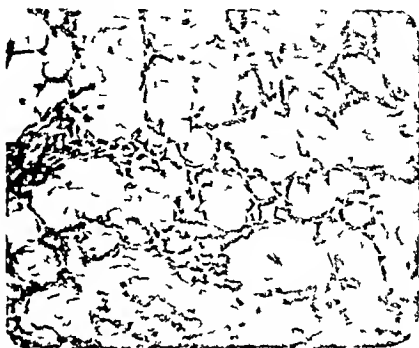


Fig. 6—Degenerative change in fibroma (lit. phas.) $\times 60$



Fig. 7—Sarcomatous degeneration of fibroma $\times 60$

In addition to the two standard types of carcinoma three tumors of the Krukenberg type were found (Fig. 8). Two of these specimens were metastatic, the primary growth of one being in the stomach and of the other in the large intestine. The morphology of both of these as well as of the third case was typical of the type. The stroma was loose and somewhat cellular and scattered throughout this stroma were collections of small acini which were lined by the typical faintly staining, signet ring shaped cells, the individual cells being distended by a secretion of mucoid character.

The third case of Krukenberg tumor occurred in a young girl and, as is usually the case, was bilateral. A second laparotomy, together with a thorough study of the gastrointestinal tract, failed to disclose a primary tumor elsewhere. Death occurred quickly and although no autopsy was obtained the case has been considered as primary in the ovary.



Fig. 21—Spindle-cell sarcoma with little stroma. $\times 250$

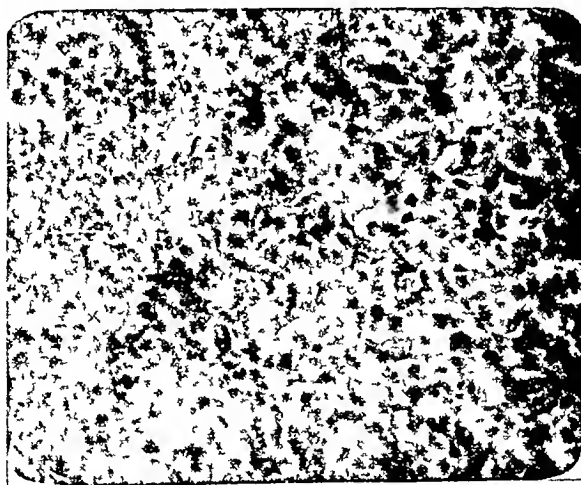
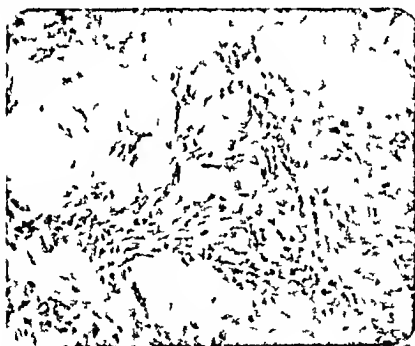
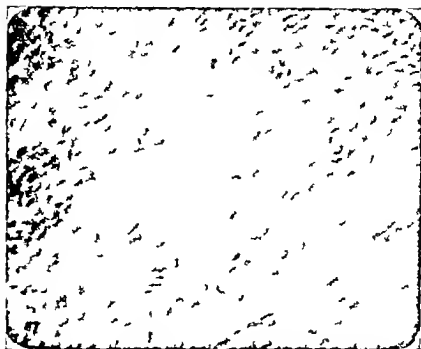


Fig. 25—Polymorphous cell sarcoma. $\times 250$

TABLE I

HISTOLOGIC CLASSIFICATION	NUMBER	PER CENT OF TOTAL
Adenofibroma	4	0.8
Papilloma	16	3.2
Fibroma	23	4.6
Sarcoma	15	3.0
Teratoma and dermoid cyst	61	12.2
Carcinoma	64	12.8
Cystadenoma	71	14.2
Simple follicle, retention, or corpus luteum cyst	246	49.5



Figs. 6 and 7—Spindle cell sarcoma with varying amounts of stroma. $\times 50$

In the series there occurred another bizarre type one instance of folliculoma ovarii. This has been elsewhere reported by Manheim.

SUMMARY

A series of 500 tumors of the ovary is presented. The incidence of the various types is given in the accompanying table.

EFFECT OF BACILLUS ACIDOPHILUS ON INTESTINAL PUTREFACTION AND INDICAN OUTPUT*

BY MAX KAHN, M A , M D , PH D , NEW YORK CITY†

INTRODUCTION

THE decomposition of proteins in the digestive tract is due chiefly to the influence of the gastric, pancreatic, and intestinal proteolytic ferments and, partly also, to the bacteria of the large intestines

INTESTINAL PUTREFACTION

Intestinal putrefactive processes depend first upon the presence of proteins and second, obviously, upon the presence of putrefactive bacteria, chiefly *Bacillus coli communis*, *Bacillus putrificus*, *Bacillus aerogenes capsulatus*. Changes in the amount of ingested proteins and changes in the virulence of the microorganisms will therefore influence putrefaction. Putrefactive bacteria are facultative, that is, they prefer carbohydrates, if available, to proteins. Thus, the amount of carbohydrates ingested will also be of influence on putrefaction.

THE ETHEREAL SULPHATES

The formation of phenol and phenolic substances (cresol, indol, skatol, etc.) has been ascribed to the action of the intestinal bacterial flora. Such organisms as the *Bacillus coli communis*, which is a normal inhabitant of the intestinal canal, are harmless under normal circumstances. In conditions of injury to the intestinal mucosa, they may become virulent (Fermi and Salto). Other organisms, like the *Bacillus putrificus* and *Bacillus aerogenes capsulatus*, which are obligatory anaerobes, thrive in the colon where there is no oxygen (Heiter), and break up protein into carbocyclic toxic substances. These substances are absorbed and, after undergoing oxidative changes, they conjugate with sulphuric acid in the liver producing nontoxic substances, the so-called ethereal sulphates. The toxicity of these bacterial products of putrefaction is well known.

Of the ethereal sulphates, indoxyl potassium sulphate, or indican, has aroused special interest and is regarded as a direct index of the total amount of ethereal sulphates conjugated, and so indicates the extent of putrefactive processes in the intestines. After having been oxidized to indoxyl and after having been conjugated with sulphuric acid and potassium, indol occurs in

*From the Laboratories Beth Israel Hospital New York City
Received for publication May 27 1926

†Director of Laboratories Beth Israel Hospital New York City Chief Diseases of Metabolism Israel-Zion Hospital New York City Associate in Biochemistry College of Physicians and Surgeons Columbia University

the urine as indoxyl potassium sulphate or indican, and the amount of indican may be taken as an index of the extent of the intestinal putrefaction.

In other investigations the amount of all of the ethereal sulphates or their relative amount in comparison with the amount of the total sulphur and the percentage of sulphate sulphur have been studied. Folin showed that indican is that part of the ethereal sulphates which is produced in the process of putrefaction. Another part of the excreted ethereal sulphates is a product of the endogenous sulphur metabolism not derived from ingested proteins.

In support of these facts it has been shown that low protein diet (Bunge Corabé), vegetarian (Hoppe Seyler) farinaceous (Rothman, Gottwald and Krauss) or carbohydrate diet (Hoppe Seyler, Poehl, Biernacki etc.), ingestion of lactose (Strauss and Philipsohn) of milk (Biernacki, Maltoda etc.) of sour milk (Poehl) and the ingestion of lactic acid bacilli (Cohen, Leav) of hydrochloric acid (Biernacki, Stadelman etc.) increase the amount of ethereal sulphates eliminated. It was also found that water drinking (Haw) and starving (Muller) decrease the amount of ethereal sulphates excreted.

REVIEW OF THE LITERATURE

Since Stadelman found phenol in cow's and horse's urine Landolt, Lieben, Hoppe Seyler, Bulgninsky and Munk found traces of it in normal human urine and Salkowski observed that in ileus and other obstructive intestinal disease, the excretion of phenol in the urine is much increased.

Baumann and Heister reported that not only phenol but also other substances were excreted in the urine as ethereal sulphates. They also observed that phenol unites not only with sulphuric acid but with other radicals. This was confirmed by Schmiedeberg who found that phenol unites with glycuronic acid. Upon poisoning dogs with phenol it was found that the liver became rich in phenol sulphates. For example in 100 parts of liver he found 19 times as much tribromphenol as in 100 parts of blood. This phenomenon seemed to prove that the liver is the seat of conjugation of the phenolic and indolic radicals with sulphuric acid.

Lang determined the quantity of ethereal sulphates in the urine of guinea before extirpation of the liver. He was led to believe that the synthesis of the ethereal sulphates was not exclusively performed in the liver.

From experiments performed *in vitro* Koch also thought that the liver was not the only seat of sulphoconjugation. He took liver, kidney, pancreas, thymus and muscle minced each organ separately and added phenol and disodium sulphate. He kept these mixtures at body temperature or else at 8° to 12° C. He reported that all the tissues except the thymus took part in the synthesis. He obtained similar results with ortho, meta and para dihydroxyphenol.

Landi repeated the experiments of Koch using only liver tissue. But due to the fact that decomposition sets in so very soon he could not confirm Koch's findings. He also made perfusion experiments with the liver and

finally assumed that the seat of conjugation of the phenolic and sulphuric radicals was not the liver but the intestines

The opinion of Lang, Koch, and Landi is directly negated by the findings of Embden and Glaessner. They performed perfusion experiments on the organs of dogs, using the liver, muscle, kidneys, lungs, and small intestines. From their investigations they concluded that the liver is the most important organ for the formation of the ethereal sulphates. Smaller quantities of ethereal sulphates are produced in the lungs and the kidneys, but the muscle tissue and the small intestine play a very insignificant rôle in the formation of the ethereal sulphates.

Beale, from his observations, was of the opinion that the liver was the seat of the synthesis of the ethereal sulphates.

Finizio confirmed Beale from his clinical findings. In normal individuals and in a case of echinococcus hepatic cyst, he found that the administration of thymol caused an increased excretion of ethereal sulphates in the urine. When, however, he administered thymol to a patient suffering from hepatic cirrhosis, he found no increase of the ethereal sulphates in the urine.

In normal conditions of the alimentary tract, Strauss and Phillipsohn found no phenol in the urine, and they concluded that, under normal conditions, the phenol and other radicals were conjugated with sulphuric acid. According to these authors, the liver is the seat of the synthesis of the ethereal sulphates.

Heiter and Waksman took 7 grams of liver, kidney, muscle, brain, and blood, respectively. These specimens were minced, and each tissue treated with 10 cc of a weak phenol solution, and allowed to stand for from two to three hours. The mixtures were then distilled, and they found that there was a loss in the phenol distilled. The liver retained most of the phenol, then came in order, the kidneys, muscle, and brain.

In conditions of jaundice, Biernacki found four times the amount of ethereal sulphates normally present. Darenberg and Percy found an increased excretion of indol and skatol in jaundiced individuals. Labbe and Vitry obtained similar results. Magiegeas obtained varying quantities of ethereal sulphates in icteric patients.

EXPERIMENTAL RESEARCH

We undertook the present study to ascertain whether it is possible to decrease the extent of intestinal putrefactive processes by means of influencing the intestinal flora. We used a culture of the *Bacillus acidophilus* for that purpose (*Vitabac*), assuming that the increasing of the acidity of the intestinal contents, as produced by the *Bacillus acidophilus*, will inhibit the growth and virulence of the putrefactive microorganisms.

Bacteriologic examination of the feces was made by Strassburger's method. A small portion of feces with water was centrifuged, the liquid part poured into another tube, diluted with an equal quantity of alcohol, and again centrifuged. From the sediment, which did not now contain anything but bacteria and yeasts, a smear was prepared and stained. The *Bacillus acidophi-*

us was identified by its Gram positive stain and its morphologic characteristics

As an index of the extent of the putrefactive processes, we determined the urinary indican sulphur. We also observed whether there occurred a decrease in the urinary indican excretion after the ingestion of the *Bacillus acidophilus*, and what changes occurred in the urinary output of the ethereal sulphates related to this decreased indicanuria.

We adopted the following technique. The patients were kept on a fixed diet. The urine was collected for two days and analyzed for total sulphur, ethereal sulphates and indican. The feces were examined bacteriologically. After taking the *Bacillus acidophilus* culture (we used that prepared by the Bergman Laboratories called *Labac*) two tablespoonsful three times daily, the urine was reexamined in three to five days and the feces analyzed as before.

Results—The total sulphur was determined by Benedict's, the ethereal sulphates by Folm's, and the indican by Ellinger's method. The urine was preserved by cold.

TABLE I

SPONTANEOUS CHANGES IN ETHEREAL SULPHATE AND INDICAN ELIMINATION

CASE NUMBER	TOTAL SULPHUR AS SO ₄ IN GRAMS		ETHEREAL SULPHATE SULPHUR AS SO ₄ IN GRAMS		INDICAN AS INDIGO IN MILLIGRAMS		ETHEREAL SULPHATE SULPHUR PER CENT OF TOTAL SULPHUR	
	FIRST	SECOND	FIRST	SECOND	FIRST	SECOND	FIRST	SECOND
	DETERMINATION		DETERMINATION		DETERMINATION		DETERMINATION	
1	19	27	0.17	0.22			87	82
2	265	23	0.15	0.15	25	25	58	34
3	48	48	0.26	0.26	89	84	55	55
4	175	235	0.20	0.23	89	188	114	98

TABLE II

ETHEREAL SULPHATE AND INDICAN ELIMINATION BEFORE AND AFTER *BACILLUS ACIDOPHILUS* ADMINISTRATION

CASE NUMBER	TOTAL SULPHUR AS SO ₄ IN GRAMS		ETHEREAL SULPHATE SULPHUR AS SO ₄ IN GRAMS		INDICAN AS INDIGO IN MILLIGRAMS		ETHEREAL SULPHATE SULPHUR PER CENT OF TOTAL SULPHUR	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
	BAC ACIDOPHILUS		BAC ACIDOPHILUS		BAC ACIDOPHILUS		BAC ACIDOPHILUS	
5	56	37	0.19	0.22	104	43	34	60
6	17	18	0.17	0.26	105	24	100	140
7	32	60	0.32	0.70	327	34	102	113
8	30					28*		
9	30	22	0.17	0.19			56	87
10	16	24	0.09	0.18			57	74
11	34	31	0.44	0.56	121	86	100	26.8
12	46	44	0.25	0.30	112	77	55	69
13		47*		0.47*		18*		100*
14	29	44	0.022	0.22	170	104	08	50
15	27	47	0.22	0.25			86	95
16	27	205	0.20	0.23	13	Traces	72	112
17	11	14	0.34	0.34	107	113	300	250
18	2.35	23	0.47	0.18	195	194	201	78
19	11	07	0.17	0.17	40	32	156	241
20	265	30	0.26	0.23			100	110
21	12	12	0.14	0.15			112	12.5

One week later

Bacteriologic examination of the feces was made in twenty cases. We found that after three to five days, the *Bacillus acidophilus* appeared in the feces. If given to the patient together with lactose, it became the dominating microorganism of the feces. Such feces appear already physically changed, the odor being less offensive, and a more rapid sedimentation of a greater mass of bacteria could be observed during and after the centrifuging. These improvements in the bacteriology of the feces persisted even two weeks after the patient stopped taking the *Bacillus acidophilus* culture. Findings similar but less pronounced were obtained when lactose was not given, but in such cases we could observe the appearance of the *Bacillus acidophilus* in the feces in smaller numbers.

Marked alterations occurred in the urinary indican output (see Table II) in all but two cases. There was a decrease in the indican excreted, ranging from 30 to 91 per cent. This decrease was already present after five days, but gradually became more pronounced if the patient continued taking the *Bacillus acidophilus* culture (see Table II, Cases 7 and 11). It is obvious that the greatest decrease of the individual output was found in cases where previously the urinary indican elimination and, as it can be assumed, the intestinal putrefaction was more marked.

According to almost all authors, the urinary indican output may be taken as an index of the extent of the putrefactive processes within the intestines. We can, therefore, also conclude from our findings that ingestion of *Bacillus acidophilus* is likely to diminish intestinal putrefaction.

As to the ethereal sulphates, our findings were unexpected. We found, along with the decrease of the indican excreted, an increase in the percentage of ethereal sulphates (and in the absolute amount) in all but the same two cases in which the decrease of the indican elimination was not obtained (see Table II). The regularity of these findings was definite. The fact that no increase of ethereal sulphates was obtained in two cases in which indican excretion was not decreased suggests a connection between these two changes—that either one is the cause of the other, or that both are the result of an underlying cause. This could only be confirmed by our findings in Case 4, Table I. In that case, the patient was kept on a fixed diet without *Bacillus acidophilus* to observe the spontaneous changes of the sulphates and of indican. We found an increase in indican output, probably due to an intercurrent enteritis. Along with this increase of indican excretion, a decrease of the percentage of the ethereal sulphates was found. Which of these is the primary change will perhaps be seen by a consideration of Case 15, Table II. In this case, in which there was no decrease in the indican output after the ingestion of *Bacillus acidophilus*, a carbuncle was present. It is well known that a pus focus in the body goes along with an increased indican production. We may assume in this case that the great part of the indican excretion was of extraintestinal origin. This emphasizes also that there is no increase in the percentage of the ethereal sulphates if indican production is not diminished. It follows from this observation that the decrease of indican produc-

tion is the primary change, inducing by some mechanism, an increase of the percentage of the ethereal sulphates

Ingestion of *Bacillus acidophilus*, by decreasing intestinal putrefaction diminished the percentage of the ethereal sulphates even separate from the factor of decrease of indican production. This is shown by the same case (see Table II). We feel that this effect of the *Bacillus acidophilus* ingestion—decreasing the percentage of ethereal sulphates—is of intestinal origin while the increase in the amount of the ethereal sulphates which follows the decrease of the indican formation, may be of an extraintestinal or metabolic origin.

Case 16, in which there was no decrease of indican output showed a similar behavior. In this case too a decrease of the percentage of the ethereal sulphates was found. As these two cases have also a previously high percentage of the ethereal sulphates the indican production may be assumed in Case 16, to be due partly to extraintestinal indican formation.

We conclude from these findings that lessened intestinal putrefaction and decrease of indican formation result from ingestion of *Bacillus acidophilus* and produce a change in metabolism increasing the percentage of the ethereal sulphates. The ethereal sulphates cannot therefore be taken as an index of intestinal putrefaction as a secondary quantitative change in the opposite direction may be caused by the lessened indican formation. In Cases 15 and 16 there was no decrease of indican formation the reduced intestinal putrefaction went along with decreased percentage of ethereal sulphates.

Our control cases, in which the urine and feces were examined at intervals of from eight to fourteen days show that without the ingestion of *Bacillus acidophilus* and without intercurrent diseases only slight changes occur in the percentage of the ethereal sulphates as well as in the indican output.

Subjectively, ingestion of *Bacillus acidophilus* produced improvement of the bowel movements, improvement of headache and a better and stronger feeling generally.

SUMMARY

After ingestion of *Bacillus acidophilus* for from three to five days this organism can be found in the feces in large number if the culture is given to the patient together with lactose and less in number if given by itself. Along with the appearance of the microorganism in the feces the urinary indican output decreased in most cases indicating a decrease in the extent of intestinal putrefaction. There are evidences which seem to show that in the formation of the ethereal sulphates two opposite effects occur in connection with the *Bacillus acidophilus* ingestion: a decrease of the "intestinal" ethereal sulphates, caused by the decrease of intestinal putrefactive processes and an increase of the "metabolic" ethereal sulphates caused by lessened indican formation. As this would explain the many contradictory findings obtained by different investigators attention should be paid to these possibilities.

REFERENCES

- Biernacky Arch f klin Med, 1891, *xliv*, 37
 Bunge Handbuch, p 315
 Cohendy Compt rend Soc de biol, 1906, *lviii*, 602
 Combe Intestinal Auto Intoxication, New York, Rebman Co, 1910, p 201
 Folin Quoted after Hawk's Physiological Chemistry
 Gottwald Chem Centrbl, 1889, *i*, 29
 Hawk Physiological Chemistry, Philadelphia, P Blakiston's Son & Co
 Hoppe Seyler Pfluger's Arch, 1882, *v*, 470, Ztschr f physiol Chem, 1889, *xii*, 31 and 36,
 Berl klin Wehnschr, 1892, 43, Habitationschr, Kiel, 1887
 Krauss Ztschr f physiol Chem, 1893, *xviii*, 167
 Leva Berl klin Wehnschr, 1908, *xlv*, 922
 Matteoda Dissertation Geneva, 1894, Jahrbuch f Tierchem, 1894, 557
 Muller Ztschr f klin Med, 1887, *xii*, 63, Berl klin Wehnschr, 1877, 433, Virchow's
 Arch, 1893, *cxvii*, 128
 Pöhl Med Wehnschr, Petersberg, 1897, 50, Maly's Jahrb d Tierchem, 1887, *xvii*, 277
 Rothman, Gottwald, and Krauss Ztschr f physiol Chem, 1869, *xli*, 16, Pfluger's Arch,
 1882, *cxix*, 525
 Kadelman Ueber den Einfluss der Alkalien, Stuttgart, 1890
 Strauss and Philipsohn Ztschr f klin Med, 1900, *xl*, 396
 Wasbutski Arch f exper Path u Pharmacol, 1889, *xxvi*, 133, 135
 For a more complete survey of the literature, see Kahn, M^{AN} Functional Diagnosis, Hagers
 town, Md, W F Prior Co, 1920

 THE ACTION OF PILOCARPIN ON THE RAT'S PUPIL*

BY J A WADDELL, M D, UNIVERSITY, VA

INTRODUCTION

PILOCARPIN was at one time regarded by many pharmacologists as distinctively a stimulant of the parasympathetic division of the autonomic system, its effect being localized specifically on the myoneural junctions. So firm was this conviction that response to it on the part of an organ or tissue was accepted as evidence of the presence of the above type of innervation. But during approximately the last decade a number of discrepancies in the narrowness of this affinity have been reported, different points of action being exhibited in certain organs of several species of animals. It now seems, as Edmunds¹ has very aptly stated, that no single tissue is attacked by pilocarpin to the exclusion of all others.

These vagaries on the part of pilocarpin have recently been reviewed by Edmunds¹ and by Sollmann.² For convenience, the most important of them may be briefly cited here as follows: (A) The parasympathetic myoneural junctions in most of the organs of the usual laboratory animals, (B) the sympathetic myoneural junctions in the uterus of the rat (Gunn and Gunn³), (C) the muscle substance of the bladder (Edmunds and Roth⁴) and the retractor penis (Edmunds⁵), and (D) the cervical sympathetic ganglia (Dale and Laidlaw⁶). In all the above, pilocarpin manifests uniformly a stimulant action, so that they are examples of transference of the point of action without alteration of the direction of action. Furthermore, in each of these cases, atropine was antagonistic and hence followed pilocarpin in the transposition

*From the Pharmacologic Laboratory of the University of Virginia
 Received for publication March 16, 1926

Up to the present time there has been presented but one instance of dissociation of the antagonism of pilocarpin and atropine (intestine of the frog, Roth⁷), and but one of an apparent inversion of pilocarpin to a depressant (cardiac vagus of the frog, Langley⁸). The latter may, however, be explained as a stimulation of the sympathetic accelerator (Sollmann). In the former, physostigmine was also transposed and dissociated from atropine antagonism. But, while pilocarpin is transposed on the frog's rectum, arecolin exhibits its usual parasympathetic action (Schuller⁹). Physostigmine, on the other hand, is the one transposed in the case of the turtle's intestine, pilocarpin and atropine maintaining their affinity for the parasympathetic (Roth¹⁰).

As the title of this article indicates this investigation is concerned with the action of pilocarpin solely on the iris of the rat. It is of interest chiefly in the presentation of an example of the caprice of this alkaloid. It may be here stated, in brief, that pilocarpin produces in the rat a mydriasis which is antagonized by physostigmine and arecolin but not by atropine, hence, it would seem to be an instance of inversion of the action of a parasympathetic stimulant or of its transposition to the sympathetic apparatus.

MATERIALS AND METHODS

Animals—The subjects observed were healthy albinos, bred in my laboratory. All ages from six months to two years were employed. Before begin-

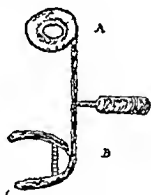


Fig. 1.—Diagram of thread counter's glass fitted with 0 mm scale for measuring the size of the rat's pupil. A the lens B the bar with the aperture which is placed over the orbit.

ning an experiment, the animals were examined for light reflex and equality of pupils.

Pilocarpin—The hydrochloride was the salt studied. The following brands were examined: Merck, Eimer and Amend, Sharp and Dohme, and Parke, Davis and Co. All produced identical effects.

Administration—Solutions of the hydrochloride were administered in the following ways: (A) Locally, into the conjunctival sac in concentrations of from $\frac{1}{8}$ per cent to 8 per cent, (B) subcutaneously into the flank, 15 mg to 4 mg per kg, and (C) intrahepatically (Waddell¹¹) 25 mg per kg.

Examination of Pupils—A thread counter's glass was employed. To the base of this a half mm scale was attached so that it could be brought very close to the pupil and the size be read off on the magnified scale. Since the lens had a very narrow focal range readings accurate to 0.1 mm could be taken. The examinations were made in diffused daylight, the light reflex was studied in direct sunlight and under a Zeiss "hammer" lamp.

Rat's Pupils—The rat's pupils are round and exceedingly small, measuring in diffuse daylight from 0.5 mm to 1 mm and in direct sunlight from 0.25 mm to 0.5 mm. While they respond readily to changes in illumination, they are not subject to such fluctuations as are observed in the cat and the rabbit due to handling and the movements of the observer.

EXPERIMENTAL DATA

The data will be considered under two captions: (A) The Pilocarpin Mydriasis, and (B) The Influence of Other Drugs and Procedures.

A. The Pilocarpin Mydriasis—Dilatation of the pupil followed pilocarpin by whatever route administered, locally, subcutaneously, and intrahepatically. Miosis was never exhibited at any stage of the experiment. Since most of the data were obtained by application to the conjunctival sac, the others being merely confirmatory, details will be given only for the local action.

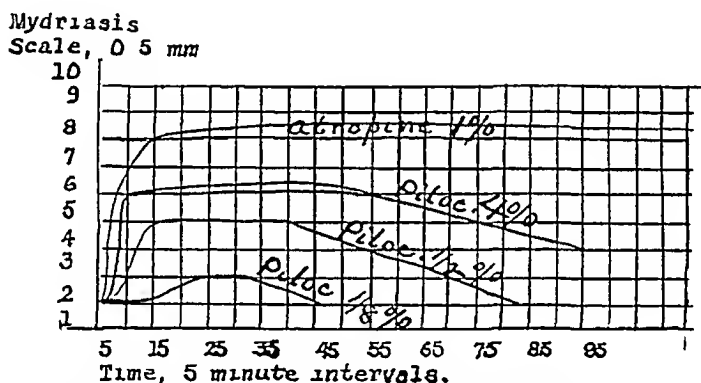


Fig. 2—Curves showing the average degree and time relations of the mydriasis after different concentrations of pilocarpine and after 1 per cent atropine.

Concentrations of from $\frac{1}{8}$ per cent to 8 per cent were all effective in producing mydriasis. There was a progressive increase in the degree, the rapidity of production, and the duration of the dilatation on increasing the strength of the solution up to 4 per cent, above that no difference was detectable. For instance, $\frac{1}{4}$ per cent dilated a 0.5 mm pupil to 1 mm and a 4 per cent to 2.5 mm, while an 8 per cent gave only 2.5 mm.

The pilocarpin mydriasis was never maximal. Atropine and asphyxia markedly increased the size of the pilocarpin pupil, as did also epinephrin and cocaine. It may be further noted here that the latter two drugs did not effect in the rat a maximal dilatation and that atropine and asphyxia also enhanced their mydriasis.

Dropped into the conjunctival sac, the lower concentrations produced no effect in the untreated (other) eye, but those of 2 per cent to 8 per cent gave a tardy and slight dilatation (absorption with resulting systemic action). Other effects were not observed in the rat, the drug apparently causing the animal no discomfort whatever. Even large doses subcutaneously did not impair the desire to eat.

Exophthalmos was appreciable with the higher concentrations of the drug but not after the lower. In cases where careful measurements were made, the widening of the palpebral fissure was by as much as 1 mm. The degree exhibited after epinephrin was marked and out of all proportion to that following pilocarpine.

No abnormalities in the position of the eyeball were noted. There was a slight photophobia, and the upper lid was perceptibly lowered in strong lights.

The pilocarpin pupil, when the full effect of the drug had been elicited, did not constrict to light but the initial stages of its mydriasis could be thus opposed. As the effect of the drug was wearing off however the light reflex was restored, for instance, a previously dilated pupil that had receded to 1 mm. could be constricted to its normal of 0.5 mm.

There is then exhibited in the rat after administration of pilocarpin a submaximal mydriasis which is not antagonized by light except in its initial and recessional stages. The phenomenon is due to a peripheral action of the drug and is accompanied by a slight degree of exophthalmos.

B The Effect of Other Drugs and Procedures—As an aid in localizing the point of action of pilocarpin on the rat's iris the behavior of the pupil to other drugs and procedures was determined. The results and their bearing on the pilocarpin mydriasis may be briefly stated as follows.

Sodium Chloride—Solutions of 1 per cent, 2 per cent and 4 per cent produced no effects. These were usually employed in the control eye during the study of the action of drugs on the other.

Carbon Dioxide—Carbon dioxide produced initially a constriction often pinpoint in degree, dilatation was not observed until a marked degree of asphyxia had been reached—usually respiratory and general paralysis—at which time the pupil suddenly and maximally dilated with loss of the light reflex. The miosis was prevented by pilocarpin, but the mydriasis was unaffected by either the prior or subsequent administration of the drug.

Epinephrin—Applied locally epinephrin did not dilate the rat's pupil but injected intrahepatically there followed instantaneously a marked degree of exophthalmos and a submaximal mydriasis with loss of light reflex. The dilatation was the same in degree as was observed after pilocarpin. The two drugs exhibited mutual summation. Extreme degrees of asphyxia however, effected even a greater degree of mydriasis than pilocarpin and epinephrin combined.

Chloral Hydrate—Chloral hydrate 0.3 gm per kg rectally, produced a pinpoint pupil, a phenomenon previously described in man and animals and ascribed to central action. This miosis can be prevented and removed by pilocarpin applied locally.

Atropine—Atropine as dilute as 0.1 per cent produced so great a degree of dilatation that only a microscopic rim of iris remained visible. There was the usual loss of the light reflex. Due to the extreme degree of mydriasis, further effects could not be detected after pilocarpin, cocaine, and other mydriatics.

Homatropine—This drug affected a maximal dilatation with loss of the light reflex. The latter observation is at variance with the results reported on other animals.¹³ On the rat, it corresponded in every way with atropine.

PROTOCOL No 1

Pupil Time	Rat No 27	Weight, 150 gm Pupil—Size in 0.5 mm		Pilocarpin—Atropine
		Poisoned	Nonpoisoned	
12 30	10		10	
Pilocarpin 4 per cent	--		Saline 4 per cent	--
12 31	10		10	
12 32	10		10	
12 33	10		10	
12 34	20		10	
12 39	30		10	
12 41	40 light		10	
12 45	50 reflex		20	
12 46	50 lost		20	
Atropine 1 per cent	--		Saline 1 per cent	--
12 48	60		20	
12 54	70		40	
12 55	80		60	
12 56	80		70	

Protocol No 1—Attention is called to the submaximal dilatation, the tardy effect on the nonpoisoned pupil, and the loss of the light reflex after pilocarpin, and to the great increase in the pilocarpin mydriasis after atropine.

PROTOCOL No 2

Pupil Time	Rat No 17	Weight, 250 gm Pupil—Size in 0.5 mm		Pilocarpin—Cocaine
		Poisoned	Nonpoisoned	
10 32	15		15	
Cocaine 2 per cent	--		Saline 2 per cent	--
10 34	20		15	
10 35	25		15	
10 37	40		15	
10 40	40		15	
Pilocarpin 2 per cent	--		Saline 2 per cent	--
10 50	60		15	
Pilocarpin 4 per cent	--		Saline 4 per cent	--
11 00	60		20	
Atropine 1 per cent	--		Saline 1 per cent	--
11 15	90		60	

Protocol No 2—Summation of the effects of pilocarpin and cocaine is shown.

PROTOCOL No 3

Pupil Time	Rat No 35	Weight, 175 gm Pupil—Size in 0.5 mm		Pilocarpin—Arecolin
		Poisoned	Nonpoisoned	
12 40	10		10	
Arecolin 2 per cent	--		Saline 2 per cent	--
12 45	02		10	
12 48	pinpoint		10	
12 50	pinpoint		10	
Pilocarpin 4 per cent	--		Saline 4 per cent	--
12 55	05		10	
12 56	10		20	
11 00	30		30	
Arecolin 2 per cent	--		--	
11 15	10		10	

Protocol No 3—The mutual antagonism between pilocarpin and arecolin is shown.

Cocaine—Introduced into the conjunctival sac, a submaximal dilatation was produced. It was like that after pilocarpin and epinephrin, with which it was additive. Atropine and asphyxia, extended the cocaine mydriasis to a maximal.

Physostigmine—One drop of a 2 per cent physostigmine solution gave a pinpoint pupil within two minutes. The untreated eye exhibited the same phenomenon after five minutes by which time well marked systemic effects were in evidence. This drug and pilocarpin were mutually antagonistic on the pupil, but not systemically so.

Arecolin—This alkaloid produced effects on the pupil identical with those following physostigmine. No systemic effects were observed on local application.

Morphine—No effect was exhibited prior to the onset of restlessness and dyspnea, at which time there was shown a dilatation (probably asphyxia). After lethal doses, a maximal dilatation like that after carbon dioxide was in evidence. In view of the above no interaction with pilocarpin could be studied.

Ergotoxin—The results with this drug were inconstant and ambiguous on both local and intrahepatic administration. Pilocarpin produced its usual mydriasis at ten, thirty, and sixty minute intervals after ergotoxin.

DISCUSSION

As a preliminary, it may be stated that the rat's pupil reacts to light, asphyxia, chloral, epinephrin, physostigmine, arecolin, cocaine and atropine in a conventional manner. Accordingly it would seem that its iris is innervated like those of other experimental animals—a constrictor mechanism with a parasympathetic nerve and a dilator with a sympathetic.

Pilocarpin applied to the conjunctival sac of one eye produces a mydriasis, initially, in that eye alone. Binocular dilatation is exhibited only after high concentrations of the drug but even then very tardily as compared with the monocular. The point of action is accordingly at the periphery, the phenomenon not being due to an indirect effect from the adrenals or asphyxia.

To further localize the point of action the evidence given by other drugs and procedures must be analyzed. A peripheral dilatation could be produced obviously by (A) stimulation of the dilator muscle or nerve with or without depression of the constrictor apparatus, (B) simultaneous but unequal stimulation of both constrictor and dilator mechanisms or (C) depression of the constrictor nerve or muscle. These possibilities will be considered in the order mentioned.

(A) Relative to the effect being due to a stimulation of the dilator structures, the following may be noted: (1) the similarity of the mydriasis by pilocarpin, cocaine and epinephrin; (2) the summation of its action with those of cocaine and epinephrin; (3) the accompanying exophthalmos, and (4) the fact that other examples of similar transposition are known. But this

evidence is not conclusive, in that it does not explain the abolition of the light reflex and of the chloral miosis. If there were, moreover, a simultaneous depression of the constrictor, a maximal mydriasis should have been exhibited, the imbalance being in such a case all in favor of dilatation. Since the observations were to the contrary, this hypothesis does not seem tenable.

(B) Simultaneous stimulation of the dilator and constrictor mechanisms would explain all the phenomena, provided there be granted a greater degree of action on the dilator than on the constrictor and provided the two opposing receptors were always affected to the proper relative degree at the same instant. It is improbable that this could occur. Should, on the other hand, pilocarpin attack (a) the constrictor sooner than the dilator, an initial miosis would have been in evidence, or (b) the dilator before the constrictor, a dilatation followed by a constriction would have been exhibited. But continuous observation failed to disclose such phenomena during the course of the pupillary action of pilocarpin, hence, the mydriasis does not seem to be due to a simultaneous stimulation of both structures.

(C) The evidence for a depression of the constrictor apparatus is as follows: (a) the blocking of stimuli from the constrictor center, demonstrated by abolition of the light reflex and the chloral miosis, (b) the agreement in direction of action with atropine and carbon dioxide, which remove the tonic constrictor impulses, (c) the antagonism to physostigmin and arecolin. The incompleteness of the pilocarpin mydriasis may be explained as indicating a weaker action than atropine, reducing but not entirely abolishing the effect of the tonically acting center, while atropine and extreme degrees of asphyxia entirely nullify the influence of the central stimuli, the former by paralyzing the nerves and the latter the center. Furthermore, the mutual antagonism between pilocarpin and physostigmin and arecolin is indicative of nervous rather than muscular depression, hence, the pilocarpin mydriasis must be attributed to a depression at the periphery of the parasympathetic nerves.

SUMMARY

1 The rat's pupil exhibits physiologically and pharmacologically conventional effects, except to pilocarpin, hence, the innervation appears to be the same as in other animals.

2 Pilocarpin dilates the rat's pupil on both local and systemic application.

3 The pilocarpin mydriasis is a peripheral phenomenon in the rat, since it is limited to the eye to which the drug is applied.

4 In the rat, pilocarpin abolishes the light reflex, the initial constriction of asphyxia, and the miosis of chloral, and hence blocks central parasympathetic impulses.

5 Pilocarpin antagonizes the effects of physostigmine and arecolin on the rat's pupil, accordingly, it affects the nervous rather than the muscular elements.

6 Pilocarpin on the rat's pupil acts like atropin but less potently.

CONCLUSIONS

Pilocarpin dilates the rat's pupil by depressing the parasympathetic constrictor apparatus at the periphery

Pilocarpin, in its effect on the rat's pupil, presents an example of inversion of the direction of action without transposition of the point of action

REFERENCES

- ¹Edmunds Jour Pharm and Exper Therap, 1922 xx 405
- ²Sollmann Physiol Rev 1922, ii, 479
- ³Gunn and Gunn Jour Pharm and Exper Therap, 1914, v 527
- ⁴Edmunds and Roth Jour Pharm and Exper Therap, 1920 xv 189
- ⁵Edmunds Jour Pharm and Exper Therap 1920, xv, 201
- ⁶Dale and Laidlaw Jour Physiol, 1912, xiv 1
- ⁷Roth Arch Internat de Pharm et de Therap 1923 xxvii 333
- ⁸Langley Jour Physiol, 1901, xxii, 237
- ⁹Schuller Arch Exper Path and Pharm 1901 xi 106
- ¹⁰Roth Jour Lab and Clin Med, 1924, ix 519
- ¹¹Waddell Jour Pharm and Exper Therap 1923 xxi 225
- ¹²Hyatt et al Jour Pharm and Exper Therap 1920 vi 415
- ¹³Macht Arch Internat de Pharm et de Therap 1922 1923 xxvii 175

STUDIES IN BLOOD GLYCOLYSIS*

GENERAL CONSIDERATION OF GLYCOLYSIS IN RELATION TO THE BLOOD CELLS AND THE PRODUCTION OF LACTIC ACID AND CARBON DIOXIDE

BY ICHIRO KATAYAMA M.D., NEW YORK CITY

A REVIEW of the literature on glycolysis warrants the conclusion that the sugar of shed blood diminishes on standing independently of bacterial contamination. Some observers¹ have stated that the sugar of diabetic blood decreases less rapidly than that of normal blood. But whether the less rapid glycolysis in diabetes is due to a different type of glucose² in the circulating blood is still a debatable question. A number of other possible factors may be responsible for this alleged difference between diabetic and nondiabetic bloods. As a preliminary to the study of the role played by the erythrocytes and leucocytes in carbohydrate metabolism, it seemed advisable to study the relation of the blood cells to glycolysis in vitro. Since these studies were begun (early in 1923), several reports have appeared in the literature on somewhat analogous experiments. Although some of the results presented in this communication are not entirely new it is believed that they are of sufficient interest to warrant their publication. The phenomenon of glycolysis has been studied in animal and human bloods and in all analyses the Folin Wu method³ was adopted for blood sugar determinations. Potassium oxalate was employed as an anticoagulant for the whole blood. The amount of the potassium oxalate used was roughly 0.06 per cent of the blood. It appeared important to con-

*From the Department of the Laboratories New York Post Graduate Medical School and Hospital.

Received for publication April 9 1926

trol this factor since Aibaia,⁴ Macleod,⁵ and Hillel, Linder and Van Slyke⁶ have reported that potassium oxalate inhibits glycolysis

In Tables I a and I b are recorded the results of studies of glycolysis in the blood of rabbits and oxen. The blood was drawn in all instances from the veins under aseptic conditions and maintained at 38° C free from bacterial contamination. Analyses of the bloods for sugar were made immediately and after three, six, and twenty-four hours. The oxen blood was re-

TABLE I a
BLOOD GLYCOLYSIS ON RABBIT*

NO	TEST HOUR	SUGAR											
		OXALATED BLOOD			CO SATURATED			SERUM			LAKED WITH H ₂ O		
		LOSS IN			LOSS IN			LOSS IN			LOSS IN		
		MG	MG	%	MG	MG	%	MG	MG	%	MG	MG	%
1	At once	139			140			150			139		
	3 hours	111	28	20	114	26	19	150	0	0	139	0	0
	6 hours	100	39	28	103	37	26	149	1	0	139	0	0
	24 hours							150	0	0			
2	At once	132			129			163			132		
	3 hours	103	29	22	115	14	11	163	0	0	132	0	0
	6 hours	97	35	27	98	31	24	163	0	0	132	0	0
	24 hours	70	62		76	53	41	163	0	0	132	0	0

*Specimens incubated at 38° C under aseptic conditions

TABLE I b
GLYCOLYSIS ON OX BLOOD IN VITRO—AT 38° C

OX NO	DURATION OF TEST	OXALATED BLOOD			DEFIBRINATED BLOOD			SERUM		
		LOSS IN			LOSS IN			LOSS IN		
		SUGAR	MG	%	SUGAR	MG	%	SUGAR	MG	%
		MG	MG	%	MG	MG	%	MG	MG	%
1	30 min	88			88			125		
	4 hours	70	18	20	75	13	15	125	0	0
	20 hours							124	1	1
2	30 min	91			91			119		
	4 hours	81	10	10	83	8	9	115	4	3
	20 hours	60	31	33	73	18	20	115	4	3
3	30 min	94			94			115		
	4 hours	85	9	10	92	2	2	115	0	0
	20 hours	72	22	23	77	17	18	115	0	0
4	30 min	100						125		
	4 hours	86	14	14				125	0	0
	20 hours	79	21	21						
5	30 min	90						134		
	4 hours	84	6	7				134	0	0
	20 hours	65	25	28				134	0	0
6	30 min	88								
	4 hours	73	15	17						
	20 hours	58	30	34						
7	30 min	125						187		
	4 hours	100	25	20				187	0	0
	20 hours	94	31	25				187	0	0
8	30 min	88						120		
	4 hours	71	17	19				120	0	0
	20 hours	70	18	20				120	0	0
9	30 min	89								
	4 hours	71	18	20						
	20 hours	41	48	54						

ceived into sterile containers when the animals were slaughtered and the sugar was determined as soon as the blood reached the laboratory (20 min) after four hours and after twenty hours. Changes in the blood sugar of the rabbits have been observed in the oxalated whole blood, in the whole blood saturated with carbon monoxide, in the blood serum, and in blood laked by dilution with water. To saturate the blood with carbon monoxide, the gas was slowly bubbled through the blood until the maximum absorption was obtained. Laking was effected by adding one volume of blood to seven volumes of water. Results are reported in milligrams of sugar per 100 cc of blood at the time of analysis.

TABLE II
BLOOD GLYCOLYSIS IN VITRO AT ROOM TEMPERATURE

CASE	AGE	SEX	TEST SPECIMEN	DURATION OF TEST				DIAGNOSIS
				At once mg	3 hours mg	6 hours mg	24 hours mg	
1 N G	50	M	Whole blood	105	91	80	22	Angina pectoris
			Serum	110	110	110	110	
			Laked blood	105	105	105	105	
2 J P	45	M	Whole blood	242	220	200		Diabetes
			Serum	277	277	268	268	
			Laked blood	242	242	242	242	
3 J M	59	M	Whole blood	176	166	154		Diabetes
			Plasma	185	185	183		
			Laked blood	176	176	176		
			CO saturated	170	166	154		
			R+ washed b c	128	125	121		
4 M G	61	M	Whole blood	105	92	78	43	Cardiac insufficiency
			Plasma	111	110	110	110	
			Laked blood	105	105	105	105	
			CO saturated	105	94	83	52	
			R+ washed b c	137	170	176	151	
5 A. A.	46	M	Whole blood	105	92	81	42	Traumatic neuritis
			Plasma	107	107	107	108	
			Laked blood	105	105	105	104	
			R+ washed b c	137	149	173	150	
6 F C	58	M	Whole blood	124	101	90		Chronic myocarditis
			Plasma	129	129	128	128	
			Laked blood	124	124	123	124	
			R+ washed b c	189	178	176	124	
7 T S	25	F	Whole blood	91	77	71	36	Hyperthyroidism
			Plasma	92	92	92	92	
			Laked blood	91	91	91	91	
			R+ washed b c	192	172	167	144	
8 W G	47	M	Whole blood	95	79	64		Teratoma testicle
			Plasma	110	109	108		
			Laked blood	95	94	95		
			CO saturated	95	79	63		
			R+ washed b c	200	197	190		
			R+ plasma	189	188	188		
9 H M	45	M	Whole blood	98	79	63		Lymphosarcoma
			Plasma	103	101	102	102	
			Laked blood	98	99	99		
			R+ washed b c	194	189	174		
			R+ plasma	182	181	181	179	

Case 3—Blood corpuscles washed with 0.9 per cent saline solution twice.

Case 4 5 6 7 8 and 9 corpuscles washed with Ringer's solution with 0 per cent glucose twice.

R = Ringer's solution with 0.2 per cent glucose

sis, and the loss of sugar is recorded in milligrams per 100 cc and as a percentage of the initial figure. During a period of six hours at 38° C the whole blood of the rabbit suffers a loss of about 27 per cent of its sugar, and after twenty-four hours about 47 per cent. Analogous changes in the blood saturated with carbon monoxide were observed. Kawashima⁷ has also demonstrated that carbon monoxide is without influence on glycolysis. There is, however, no loss of sugar in the blood serum or in the laked blood. Glycolysis was observed in oxen blood, here, however, the disappearance of the sugar was less rapid. In 8 of the 9 cases after twenty hours at 38° C the loss of sugar amounted to 20 to 34 per cent of the original concentration. In the last experiment a loss of 54 per cent after twenty hours was noted. In the defibrinated oxen blood a fall of sugar similar to the oxalated blood was seen, but in the serum the sugar remained unchanged. The apparent fall of 1 per cent in Case 1 and 3 per cent in Case 2 is no doubt due to analytical errors. This fall in blood sugar is not due to activity of microorganisms, since the specimens were cultured at the end of incubation and gave no bacterial growth.

Table II presents data on glycolysis in human blood. Nine cases were studied, and of these 2 were diabetics, 1 a hyperthyroid, and the remainder miscellaneous cases with no evident disturbance of carbohydrate metabolism. In all instances a continuous decrease of the sugar of the whole oxalated blood is observed after three, six, and twenty-four hours at room temperature, but the sugar of the serum, plasma and laked blood remain unchanged. In Cases 3, 4, and 8 the drop in the sugar of the blood saturated with carbon monoxide parallels that of the oxalated blood. From these observations on rabbit, oxen and human bloods, the conclusion appears warranted that the presence of the intact blood cells is essential for glycolysis. To substantiate this opinion, in Case 3 the erythrocytes were removed by fractional centrifugation in narrow tubes and washed with 0.9 per cent NaCl solution. The red cells were then suspended in four times then volume of Ringer's solution containing 0.2 per cent of Kahlbaum's glucose. This mixture was allowed to stand for twenty-four hours at room temperature, and determinations of the blood sugar were made at intervals of three, six, and twenty-four hours. A drop in the sugar concentration of the mixture was found, but the decrease is less than that observed in the whole blood. In Cases 4, 5, 6, 7, 8, and 9 similar experiments were conducted except that the blood cells were washed with the Ringer's solution containing the glucose in place of the 0.9 per cent NaCl. A decrease of the sugar was found in all instances, however, the loss of sugar is less than that reported for the corresponding whole bloods. In 8 and 9 the glycolytic action of the plasma towards the sugar contained in the Ringer's solution was examined. In these 2 experiments the volume of plasma used corresponded to the volume of the blood cells in the former experiments. No change was noted in the concentration of the sugar of the mixture after standing three, six, or twenty-four hours.

A comparison of the glycolytic action of the blood cells upon the blood sugar as controls, and upon the sugar added to Ringer's solution has been made in 8 cases, of whom 3 were diabetics. The results of these experiments are

recorded in Table III. The whole oxalated blood was allowed to stand at 38° C under aseptic conditions for twenty four hours. Determinations of the sugar were made immediately and at three, six and twenty four hour intervals. A fall in blood sugar is observed in all cases. As in the previous experiments the erythrocytes of the corresponding bloods were separated by centrifugation, washed with physiologic salt solution and suspended in 4 times their volume of Ringer's solution containing 0.2 per cent glucose. These mixtures were analyzed for sugar simultaneously with the whole bloods. The hemoglobin concentrations of the whole bloods are reported as indices of the cell volumes. In the final column the loss of sugar in milligrams per 100 cc is recorded. The sugar concentration is diminished both in the whole bloods and in the mixtures of erythrocytes and Ringer's solution. In Case 8 the whole blood and the erythrocytes in Ringer's solution were maintained for twenty four hours under anaerobic conditions. In this instance the whole blood during twenty four hours under aerobic conditions lost 70 mg. and during the same period under anaerobic condition lost 65 mg. per 100 cc. The loss in sugar in the mixture of erythrocytes and Ringer's solution is comparable with losses in the other cases under aerobic conditions. The loss of sugar in these mixtures cannot be explained by the glycolysis of the sugar contained in the red cells alone. The maximum concentration of sugar in the erythrocytes was found to be 0.024 per cent. Since the cells formed but one fifth of the total volume of the mixtures, it is evident that if all the sugar of the erythrocytes disappeared, the loss would not exceed 5 mg. per 100 cc. The sugar loss however, varies from 25 to 39 mg., hence it is obvious that a portion of the glucose of the Ringer's solution no longer reacts as glucose. In the nondiabetic bloods the loss of sugar is not greater than that of the diabetics. Of the three diabetics, Case 4 had received insulin but Cases 3 and 5 had received no insulin before drawing the blood. It is observed that the rate of glycolysis in Cases 3 and 5 is greater than that seen in 4. There appears to be no demonstrable difference in the rate of glycolysis in either diabetic or hyperthyroid and normal bloods. Macleod,² Macleod and Pearce³ and Cajon and Crouter⁴ have been able to demonstrate no significant variation in the glycolysis of diabetic and nondiabetic bloods. It is also interesting to note that the blood cells of diabetic bloods when incubated with Ringer's solution containing glucose produce a loss of sugar as great as that observed with nondiabetic blood cells. A quantitative comparison of the glycolytic action of the erythrocytes in the whole blood and in Ringer's solution cannot be made from these data since no attempt was made to preserve the same volume of erythrocytes in the Ringer's solution as that found in the corresponding whole bloods.

A comparison of the rate of glycolysis effected by the blood cells in their plasma and in Ringer's solution containing glucose has been made in Table IV. The blood cells were washed once with physiologic salt solution and then added to 4 times their volume of Ringer's solution containing 0.2 per cent glucose and in four times their volume of plasma. The volume of cell suspension used was adjusted so that the cell count was about 23 million per cu. mm. and the leucocyte count about 220 per cu. mm. Sugar concentrations were determined

TABLE III

BLOOD GLYCOLYSIS IN VITRO ON WHOLE BLOOD AND WASHED BLOOD CORPUSCLES IN RINGER'S SOLUTION WITH 0.2 PER CENT GLUCOSE AT 38° C

CASE	AGE	SEX	TEST SPECIMEN	At once mg	DURATION OF TEST				Loss in mg	Hb gm per 100 cc	DIAGNOSIS
					3 hours mg	6 hours mg	24 hours mg				
1 E P	19	F	Whole blood R + B C	65 170	49 156	35 150	17 117	32 39	10.3		Hyperthyroidism
2 C C	21	F	Whole blood R + B C	87 170	66 159	41 153	18 122	48 37			Auricular fibrillation
3 R E	60	F	Whole blood R + B C	176 172	158 155	125 150	49 127	109 28	11.3		Diabetes
4 P G	41	F	Whole blood R + B C	125 172	91 156	70 148	28 124	63 32	12.6		Diabetes
5 E B	62	F	Whole blood R + B C	185 170	163 155	138 148	72 123	91 32	13.2		Diabetes
6 M P	50	F	Whole blood R + B C	105 170	74 158	50 151	25 132	49 26	8.3		Carcinoma cervix
7 M M	35	F	Whole blood R + B C	90 172	70 159	52 153	26 134	44 25	12.7		Fibroid of uterus
8 G R	50	M	Whole blood Anaerobic R + B C	134 135 169	106 114 161	71 84 154	36 49 135	70 65 26	14.5		Alkalosis CO ₂ P = 92 P _H = 7.6

R + B C = Washed blood corpuscles in Ringer's solution with 0.2 per cent glucose

Hb = Hemoglobin

Case 3 and 5—no insulin treatment

Case 4—insulin treatment

Blood corpuscles washed once with 0.9 per cent saline solution

Sugar content in washed blood corpuscles = 0.024 per cent.

immediately and after twenty four hours. The Ringer's solution had a P_H of 7.35. The loss of sugar from the Ringer's solution in all instances was greater than that from the plasma, however, the initial sugar concentration was higher in the Ringer's solution than in the plasma. Continuous washing of the blood cells with 0.9 per cent sodium chloride solution diminishes the rate of glycolysis when these cells are added to Ringer's solution containing glucose. It is seen in Table V a that the amount of sugar lost during twenty four hours incubation at 38° C, progressively diminishes with the number of washings of the blood cells in physiologic salt solution.

The rate of glycolysis appears dependent upon the number of blood cells present in the incubated mixture. In Table V b there is a comparison of the

TABLE IV
GLYCOLYSIS ON WASHED BLOOD CORPUSCLES IN PLASMA AND IN RINGER'S SOLUTIONS CONTAINING 0.2 PER CENT GLUCOSE INCUBATED AT 38° C

CASE	DURATION OF TEST	1 C C WASHED BLOOD CORPUSCLES ADDED TO 4 C C RINGER'S SOLUTION		1 C C WASHED BLOOD CORPUSCLES ADDED TO 4 C C PLASMA	
		mg	Loss in mg	mg	Loss in mg
1 L C	At once	176		80	
	24 hours	131	55	42	38
2 D T	At once	182		97	
	24 hours	140	36	67	30
3 C G	At once	176		91	
	24 hours	129	47	51	40
4 A. M	At once	174		63	
	24 hours	129	46	30	33
5 R R	At once	176		100	
	24 hours	129	47	67	33
6 I. R.	At once	172		63	
	24 hours	129	43	40	33
7 C H	At once	170		83	
	24 hours	125	45	53	30
8 M. M	At once	172		135	
	24 hours	120	52	97	38

Ringer's solution $P_H = 7.35$

Blood corpuscles washed once with 0.9 per cent NaCl solution

Blood corpuscles suspended both in Ringer's solution and in plasma.

Erythrocytes average 2,300,000 per cu mm

Leucocytes average 220 per cu mm

rate of glycolysis in mixtures in which the washed blood cells were suspended in 4 times and in 15 times their volume of Ringer's solution containing glucose. In the former mixtures the erythrocytes averaged 2.3 million and the leucocytes 220 per cu mm, while in the latter mixtures the erythrocytes were about 4.5 million and the leucocytes 500 per cu mm. In the latter mixtures the initial sugar concentration is less than in the former but the amount of sugar lost within twenty four hours is more than double that lost in the mixtures containing the smaller number of blood cells. Macleod¹⁰ concludes from his numerous experiments on glycolysis that it is an intracorpuseular process. Levine and Meyer¹¹ have shown that when leucocytes were suspended in Henderson's phosphate solutions containing glucose, a portion of the glucose, as such, disappeared with the production of lactic acid. It is difficult to say in the experiments re-

Specimens of whole blood from 5 individuals, 2 cases of hyperthyroidism and 3 of diabetes, were divided into 3 groups. One group of specimens was maintained in hot air incubator at 38°C , the second group was permitted to stand at room temperature ($20^{\circ}\text{--}22^{\circ}\text{C}$), and the third was placed in the ice box at 4°C for twenty-four hours. The sugar concentrations of all specimens were determined at intervals of three, six, and twenty-four hours. Table VII gives the data on the sugar concentrations found, and the loss in sugar expressed in milligrams per 100 cc and in per cent of the original figure. For all three periods of incubation the maximum loss occurs in the blood maintained at 38°C and the minimum in those preserved in the ice box. At a temperature of 38°C the loss in sugar after twenty-four hours expressed as per cent of the original concentration varied from 42 to 83 per cent, at room temperature from 23 to 78 per cent and in the ice box from 2 to 33 per cent. The rate of glycolysis appears to vary directly with the temperature to 38°C . It is of practical importance to note that preserving blood in the ice box does not prevent a loss of sugar.

A comparison of glycolysis before and after the production of a hyperglycemia by the ingestion of glucose has been made in the experiments recorded in Table VIII. It is seen that after twenty-four hours at 38°C in Case 1 the blood has lost 92 mg per 100 cc of sugar, this loss amounting to 83 per cent of the original concentration. The ingested glucose produced a blood sugar of 195 mg per 100 cc. During twenty-four hours, 155 mg of sugar for every 100 cc are lost from this blood, representing 79 per cent of the original concentration. In the hyperglycemic blood the loss expressed in milligrams per 100 cc is greater than in the control blood, but expressed as a fraction of the original amount of sugar, it is equivalent to that of the control. In the second case the intake of glucose has raised the blood sugar from 93 to 120 mg per 100 cc. In both specimens of blood 80 mg of sugar per 100 cc of blood are lost after twenty-four hours at 38°C . The loss after the glucose ingestion expressed as per cent of the original concentration is less than that of the control blood.

The addition of insulin to whole blood during the period of incubation was without effect upon the rate of glycolysis. It is seen in Table IX that the loss of sugar at 38°C in the specimens of blood to which insulin had been added (10 units per 10 cc blood) parallels the loss of sugar in the control specimens.

Evans¹³ has observed a fall in the carbon dioxide capacity of shed blood which, he believes, is due to a conversion of glucose into lactic acid as a result of glycolysis. Kraske¹⁴ in his studies on seven human subjects reported a quantitative conversion of the lost blood sugar into lactic acid during an incubation of two hours. Kondo,¹⁵ however, in his experiments on glycolysis in dogs' blood during a similar period of incubation found that the increase in lactic acid was much less than the amount of sugar lost. Von Noorden¹⁶ has produced evidence to show that the lactic acid formed is d-lactic acid. Morgulis and Barkus¹⁷ have attempted to emphasize the difference between glycolysis *in vitro* and the glycolysis within the organism after insulin on the basis of their observations that in the former the disappearance of the sugar goes parallel with a formation of lactic acid. Their figures, however, do not demonstrate a parallel rise in lactic

TABLE VII
BLOOD GLYCOLYSIS IN VITRO AT DIFFERENT TEMPERATURES

CASE†	AGE	SEX	DURATION OF TEST	38° C. INCUBATED			ROOM TEMPERATURE			ICE BOX			DIAGNOSIS
				Sugar mg	Loss mg	per cent	Sugar mg	Loss mg	per cent	Sugar mg	Loss mg	per cent	
1 R R	28	F	At once 3 hours 6 hours 24 hours	91 61 40 17	30 51 74 74	33 56 81 81	91 72 64 20	19 27 71 71	21 30 78 78	91 89 88 78	2 3 13 13	2 3 14 14	Hyperthyroidism
2 J E	25	F	At once 3 hours 6 hours 9 hours 24 hours	110 81 56 36 18	29 54 74 92 92	26 49 67 83 83	110 89 76 55 40	21 34 55 70 70	19 31 50 64 64	110 99 9 88 74	11 17 22 36 36	10 15 20 33 33	Hyperthyroidism
3 P G	41	F	At once 3 hours 6 hours 9 hours 24 hours	434 400 370 341 251	34 64 93 132 132	8 15 22 42 42	434 417 406 400 334	17 28 34 100 100	4 6 8 23 23	434 422 416 410 300	12 17 18 24 9	3 4 5 6 3	Diabetes*
4 A G	42	F	At once 3 hours 6 hours 24 hours	300 261 252 166	39 48 134 134	13 16 45 45	300 201 230 230	30 46 70 70	13 15 23 23	291 291 291 298	9 9 9 02	3 3 3 21	Diabetes*
5 E G	63	F	At once 3 hours 6 hours 24 hours	176 138 131 76	38 45 100 100	22 20 57 57	176 153 144 105	23 32 71 71	13 18 40 40	176 174 174 172	2 2 2 4	1 1 2 2	Diabetes*

Insulin treatment.

Ice box disordered (not enough cold)

†Specimens cultured after standing twenty four hours no bacterial growth

TABLE XII

CHANGING OF SUGAR AND CO₂ CONTENT OF SHED BLOOD ON STANDING IN INCUBATOR AT 33° C
UNDER ANAEROBIC CONDITIONS

CASE	AGE	SEX	SPECIMENS	TEST HOUR		LOSS IN VOLUME
				At once	24 hours	
1 G I	38	F	Blood sugar (mg)	183	66	117
			CO ₂ content of plasma (vol per cent)	55 0	53 0	2
2 J P	16	M	Blood sugar (mg)	103	38	65
			CO ₂ content of plasma (vol per cent)	63 3	60 0	3 3
3 H R	15	F	Blood sugar (mg)	157	71	86
			CO ₂ content of plasma (vol per cent)	65 0	55 1	9 9
4 M K	26	M	Blood sugar (mg)	189	85	104
			CO ₂ content of plasma (vol per cent)	55 1	53 2	1 9
5 M A.	31	F	Blood sugar (mg)	136	44	92
			CO ₂ content of plasma (vol per cent)	56 6	52 8	3 8
6 Y K	52	F	Blood sugar (mg)	306	142	164
			CO ₂ content of plasma (vol per cent)	58 5	54 7	3 8
7 H S	24	M	Blood sugar (mg)	104	24	80
			CO ₂ content of plasma (vol per cent)	59 1	50 7	8 4
8 W M	8	M	Blood sugar (mg)	114	18	96
			CO ₂ content of plasma (vol per cent)	57 2	48 8	8 4

TABLE XIII

CHANGES IN GLUCOSE AND GAS CONTENT ON WASHED BLOOD CORPUSCLES IN RINGER'S SOLUTION AND PHYSIOLOGIC SALT SOLUTION CONTAINING GLUCOSE UNDER ANAEROBIC CONDITIONS AT 33° C

CASE	TEST SPECIMENS	SUGAR			VOL. OF GAS		ERYTHROCYTES million per cu mm	LEUCOCYTES per cu mm
		At once	24 hours after	Loss	At once	24 hours after		
1 F M	Blood corp in R + 0.2 per cent glucose	160	112	38	1.2	1.4	2.3	120
	Blood corp in R + 0.1 per cent glucose	85	53	32	1.3	1.3	1.8	310
	Blood corp in 0.9 per cent NaCl sol + 0.2 per cent glucose	165	135	30	1.3	1.3	1.8	430
2 F N	Blood corp in R + 0.2 per cent glucose	156	118	38	1.1	1.1	2.3	210
	Blood corp in 0.9 per cent NaCl sol + 0.2 per cent glucose	154	133	21	1.1	1.1	2.4	300
	Blood corp in R + 0.2 per cent glucose	158	135	23	1.1	1.2	2.3	180
3 R B	Blood corp in 0.9 per cent NaCl sol + 0.2 per cent glucose	161	135	26	1.0	1.0	2.3	330
	Blood corp in R + 0.2 per cent glucose	170	114	56	1.2	1.4	2.5	180
	Blood corp in 0.9 per cent NaCl sol + 0.2 per cent glucose	176	121	55	1.1	1.1	2.5	120
4 Rabbit	Blood corp in R + 0.2 per cent glucose	167	115	52	1.1	1.3	2.3	200

Total volume = 10 c.c. each test
Sugar expressed in mg per 100 c.c.

cent, and after twenty four hours from 17.9 to 24.1 volumes per cent. The production of a nonvolatile acid must be responsible for this depletion in the alkaline reserve although Mellanby and Thomas believe that the fall in the alkali reserve of the blood plasma during glycolysis is due to the lactic acid produced. The amount of lactic acid formed during glycolysis (Table X) is sufficient to account for but a small fraction of the decrease in alkaline reserve noted in Table XI. Evans¹² has shown that this decrease in the carbon dioxide capacity of the shed blood proceeds with a progressively diminishing velocity. From the figures reported in Table XI, it is evident that from $\frac{1}{3}$ to $\frac{1}{2}$ of the total decrease occurs within the first four hours of incubation. It appears then that other fixed acids in addition to lactic acid must be formed during glycolysis.

During anaerobic glycolysis at 38° C. for twenty four hours there is little change in the carbon dioxide content of the blood plasma (Table XII). In the 8 experiments tabulated, the blood sugar decrease varied from 65 to 164 mg., the greatest decrease was observed in the specimen showing a hyperglycemia (306 mg.) immediately after withdrawal. The fall in carbon dioxide content ranged from 1.9 to 8.4 volumes per cent. This decrease in the carbon dioxide content may be explained by a loss of the gas into the oil at the temperature of incubation. Association of the data in Tables X and XI demonstrate that the carbon dioxide, released from the plasma bicarbonate during the neutralization of the acids produced in glycolysis, is retained in the oil covered blood. Apparently glycolysis does not result in a production of carbon dioxide from the glucose lost.

The experiments reported in Table XIII were planned to obtain further information on this point. Blood cells washed once with 0.9 per cent NaCl solution were placed in Ringer's solution containing 0.1 or 0.2 per cent glucose. In all cases the total volume of the mixture was 10 c.c. and incubation was carried out under anaerobic conditions. A similar loss of sugar is noted in the suspensions of blood cells in Ringer's solution and in physiologic salts solution. It is of interest to note also that in Experiment 1 the loss in sugar from the Ringer's solution containing 0.1 per cent glucose is 3.2 mg. whereas the loss from solution containing 0.2 per cent glucose was 3.8 mg. The proportion of cells to the solutions was adjusted so that the erythrocyte content of all mixtures would be fairly close. The erythrocyte content varied from 1.8 to 2.5 million per cu. mm., and the leucocytes from 120 to 430 per cu. mm. The total volume of gas in the mixtures before and after incubation appear unchanged. The volume of gas was determined on the centrifuged specimens by following the technique for CO₂ content. The volumes of the gas were measured at from 20° to 25° C. and although these volumes have not been corrected to reduce them to volumes of CO₂, it is evident that since the volumes remained practically unchanged, no production of CO occurs as a result of glycolysis.

SUMMARY

The sugar of shed blood gradually decreases on standing without bacterial contamination and under either aerobic or anaerobic conditions. This decrease is greatest at 38° C. and least in the ice box.

Plasma, serum and hemolysed blood show no loss of sugar on standing
Saturation of whole blood with carbon monoxide does not inhibit glycolysis

When washed blood cells are added to Ringer's solution or to physiologic salt solution containing glucose, fructose or galactose, glycolysis occurs. The decrease in sugar concentration is greatest with glucose and least with galactose.

The rate of glycolysis is dependent upon the blood cell volume.

There appears to be no demonstrable difference between the rates of glycolysis in diabetic and nondiabetic bloods. Furthermore the blood cells of diabetic bloods cause as rapid a rate of glycolysis in Ringer's solution as the cells of nondiabetic bloods.

Insulin therapy or insulin *in vitro* has no effect upon the rate of glycolysis.

The decrease in sugar concentration is accompanied by a production of lactic acid, but the increase in lactic acid does not account for the total amount of sugar lost.

Other acids than lactic acid are evidently produced during glycolysis. There is no production of carbon dioxide.

Note—The writer's thanks are due to Dr. John A. Killian for his constant advice and very essential help during the course of this work.

REFERENCES

- ¹Denis, W., and Giles, U. *Jour Biol Chem*, 1923, lvi, 739
- ²Winter, L. B., and Smith, W. *Brit Med Jour*, 1923, i, 710
- Forrest, W. D., Smith, W., and Winter, L. B. *Jour Physiol*, 1922-1923, lvi, 224
- ³Folin, O., and Wu, H. *Jour Biol Chem*, 1920, xli, 367
- ⁴Aibara, C. *Jour Biochem*, 1922, i, 457
- ⁵Macleod, J. J. R. *Jour Biol Chem*, 1913, xv, 497
- ⁶Hiller, A., Linder, G. C., and Van Slyke, D. D. *Jour Biol Chem*, 1925, lxxv, 62
- ⁷Kawashima, Y. *Jour Biochem*, 1923, ii, 131
- ⁸Macleod, J. J. R., and Pearce, R. G. *Am Jour Physiol*, 1913, xxxii, 184
- ⁹Cajori, F. A., and Crouter, C. Y. *Jour Biol Chem*, 1924, lx, 765
- ¹⁰Macleod, J. J. R. *Physiol Rev*, 1921, i, 208
- ¹¹Levine, P. A., and Meyer, G. M. *Jour Biol Chem*, 1912, vi, 361
- ¹²Osnato, M., and Killian, J. A. *Arch Neurol and Psychiat* (in press)
- ¹³Evans, C. L. *Jour Physiol*, 1922, lvi, 146
- ¹⁴Kraske, B. *Biochem Ztschr*, 1912, xlv, 81
- ¹⁵Kondo, K. *Biochem Ztschr*, 1912, xlv, 88
- ¹⁶Von Noorden, K. *Biochem Ztschr*, 1912, xlv, 94
- ¹⁷Morgulis, S., and Barkus, O. *Jour Biol Chem*, 1925, lxxv, 1
- ¹⁸Briggs, A. P., Koechig, I., Doisy, C. A., and Weber, C. J. *Jour Biol Chem*, 1924, lxxiv, 721
- ¹⁹Tolstoi, E., Loevel, R. O., Levine, S. F., and Richardson, H. B. *Proc Soc Exper Biol and Med*, 1923-1924, xxi, 449
- ²⁰Katayama, I., and Killian, J. A. *Proc Soc Exper Biol and Med*, 1925, xxiii, 173
- ²¹Clausen, S. W. *Jour Biol Chem*, 1923, lxi, 263
- ²²Mellanby, J., and Thomas, C. J. *Jour Physiol*, 1920-1921, lv, 178

A NOTE UPON THE PRESERVATION OF SHEEP CELLS FOR USE IN COMPLEMENT FIXATION TESTS*

By ROBERT A. KILDUFFE,† M.D., ATLANTIC CITY, N. J.

WHILE freshly collected cells are to be preferred for the conduct of complement fixation tests it frequently becomes necessary, under certain circumstances or combinations of circumstances, to resort to some method of preserving blood for varying periods.

This contingency may confront small laboratories or even large ones in which the keeping of laboratory sheep is impracticable for one reason or another. Abattoir blood, for example, may not be obtainable at regular or unvarying intervals, or as has happened, foot and mouth disease or other epidemic condition may interfere with the source or regularity of the supply.

In common with many other workers, for reasons beyond immediate control, I have used abattoir blood for the preparation of cell suspensions for complement fixation tests, and in order to lessen the frequency of collection the blood has been preserved. Such emergencies, as for example quarantine or embargo, have occurred in the past, which, without a satisfactory method of preservation, would have occasioned some annoyance and great inconvenience.

The method of preservation adopted and in use for some years is neither original nor new. It has been so satisfactory however that in view of the number of methods which have been proposed (thus arguing for imperfections in all), attention is again called to it in this note.

There are several details essential for success. Unless the possibility is recognized and due precautions are taken, blood may be collected in pails or other abattoir containers and transferred to the laboratory jars as called for, and as may happen, when the blood so collected stands for some time, or perhaps, because the container may be hastily or carelessly cleaned the blood on its arrival in the laboratory will not be fit either for immediate use or for preservation.

The procedure followed is given here in detail. An ordinary quart size Mason jar is thoroughly cleaned and sterilized by dry heat. A sufficient quantity of 10 per cent sterile sodium citrate in normal saline is added to make a layer about one inch deep in the jar. The jar and its contents are then autoclaved.

One of these jars, by previous arrangement, is kept at the abattoir and filled directly from the animal when the sheep is killed and a new container is left in its place when the blood is collected.

Immediately upon arriving in the laboratory the blood is preserved by the

*From the Laboratories of The Atlantic City Hospital.

Received for publication, April 30 1926.

†Director Laboratories Atlantic City Hospital.

following method To each 80 c c of blood is added 1 c c of a 1:10 dilution of formalin in normal saline after the method described by Bernstein and Kaliski.¹

A convenient quantity—240 c c or 320 c c—of the preserved blood is placed in a clean, dry, glass-stoppered bottle which is kept in the ice chest.

Cells are perfectly satisfactory for use for a period of two weeks after preservation by this method. While it is the custom in this laboratory to collect blood at weekly intervals, blood has been preserved and used in emergency for as long as twenty-seven days.

According to Kolmer,² the blood is not considered fit for use unless the following conditions are fulfilled:

1. Absence of discoloration of the supernatant fluid after the second washing with normal saline.

2. Return of the normal bright red color on washing.

Blood so collected and preserved has been found satisfactory, in emergency, for the preparation of blood plates.

The method is simple, rapid, inexpensive, eminently satisfactory, and deserves a wide circulation.

REFERENCES

- ¹Bernstein, E. P., and Kaliski, D. J. The Use of Formalized Sheep Cells in Complement Fixation Tests, *Ztschr. f. Immunol.*, 1912, *vi*, 490.
- ²Kolmer, J. A. Infection, Immunity, and Biologic Therapy, Ed. 3, Philadelphia, Pa., W. B. Saunders Co., p. 13.

THE INFLUENCE OF DIET ON THE PHYSIOLOGIC ASSAY OF INSULIN*

By DR. A. STASIAK (BUDAPEST)

VARIOUS authors have investigated the effect of diet on the susceptibility of animals to insulin. Macleod and coworkers¹ observed that the blood sugar in starved rabbits did not recover as rapidly as in fed rabbits, following the hypoglycemia due to insulin. Page² showed that rabbits on a diet of oats and bread, which produces slight acidosis, are very resistant to insulin. These results were confirmed by Blatherwick and coworkers,³ who found that rabbits kept on a diet poor in carbohydrates were more resistant to insulin than were animals well fed with carbohydrates.

Tutso⁴ found that the initial fall in blood sugar after insulin injection in rabbits was more pronounced after carbohydrate feeding than after prolonged starvation. Abderhalden and Wertheimer,⁵ using white rats, also found these animals most susceptible to insulin on a diet rich in carbohydrates.

The following experiments were performed in order to investigate the

*From the Insulin Committee Laboratory, University of Toronto, Toronto, Canada.
Received for publication May 2, 1926.

influence of diet on the results of the physiologic assay of insulin as ordinarily conducted

Rabbits were well fed with carrots, hay, and oats, the food being removed fifteen minutes before the commencement of the experiments. As controls, rabbits which had been starved for twenty four hours were used. All the animals were injected with the same dose of the same insulin (designated Standard S 6), the dose being 2 units per 2 kg body weight. Blood samples were taken before injection and one and a half, three, and five hours after injection. The blood sugar was determined by the method of Shaffer and Hartmann,⁶ and the assays were calculated by the formula given by Macleod and Orr.⁷

The average lowering of the blood sugar ("a" in the formula) was 0.033 per cent in the case of the 18 fed animals used and 0.031 per cent in the

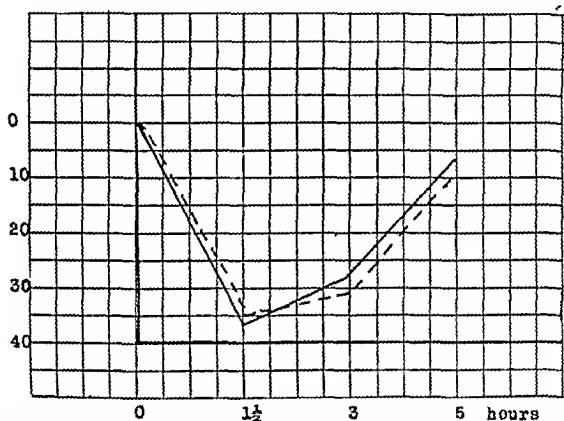


Fig 1—Shows the fall in blood sugar expressed in mg per cent produced by the injection of 2 units per 2 kg and observed at the times shown. The solid line represents the fall in fed animals and the broken line the fall in starved animals.

case of the 18 starved animals, the corresponding assay results being 10.2 units per cc and 12.1 units per cc, respectively.

In the accompanying table we give the average values of the normal blood sugar and of the blood sugar one and a half, three and five hours after the injection of insulin. From these figures we have calculated the percentage fall of the blood sugar at one and a half, three, and five hours in relation to the normal blood sugar.

The tabulated results are plotted in the figure. A few observations were also undertaken to study the effect of fasting and carbohydrate feeding on the initial fall in blood sugar following insulin. The striking increased sensitivity of carbohydrate fed animals, described by Tutso and others, was not observed to occur. This may have been due to the occurrence of a temporary increase in blood sugar in the fasting animals immediately following the injection of insulin.

TABLE I

	NORMAL %	1½ HOURS %	3 HOURS %	5 HOURS %	"a"	ASSAY IN UNITS
<i>Average for 18 fed animals</i>	0 139	0 088	0 102	0 130	0 033	10 2
Percentage fall in blood sugar	0	37	28	7		
<i>Average for 18 starved animals</i>	0 120	0 077	0 083	0 109	0 031	12 1
Percentage fall in blood sugar	0	36	31	9		

CONCLUSION

It would rather appear, from the above results, that the actual range through which the blood sugar is lowered by insulin is approximately the same in fed rabbits and in rabbits from which food has been withheld for twenty-four hours

REFERENCES

- ¹Macleod, J J R, et al Jour Physiol, 1923, lvi, 234
- ²Page, T H Am Jour Physiol, 1923, lvi, 1
- ³Blatherwick, N R, et al Am Jour Physiol, 1924, lvi, 155
- ⁴Tutso, M Proc Soc Exper Biol Med, 1925, xiii, 40
- ⁵Abderhalden, E, and Wertheimer, E Arch f d ges Physiol, 1924, cxi, 439
- ⁶Shaffer, P A, and Hartmann, A F Jour Biol Chem, 1920 1, vi, 365
- ⁷Macleod, J J R, and Orr, M D JOUR LAB AND CLIN MED, 1924, ix, 591

A NOTE ON THE COMPLEMENT FIXATION REACTION IN ANTIPOLLEN SERUM*

By SUSAN GRIFFITH RAMSDALL, M A, Los Angeles, Calif

WHETHER the allergic states in man have a basis in common with the condition resulting from artificial immunization in animals may still be a debatable question. But success in demonstrating certain definite immunologic phenomena—precipitins, complement fixation, shock, skin reaction, Schultz Dile reaction,—following treatment of laboratory animals with pollens or pollen extracts, has been reported many times. The failures to confirm the findings and the scarcity of experimental data in many of the reports have, however, left enough shadow of doubt upon the status of the test in this connection to seem to warrant a report with a description in some detail of the special difficulties involved of another effort to secure complement fixation in the sera of rabbits treated with pollen.

In this laboratory the greatest single need was for a standard method of preparation, stabilization, and measuring the antigenic content of the solutions of pollens with which patients were to be treated for the purpose of desensitization. As to the reported methods of preparation, each offered a particular advantage: one that the protein extraction was more complete, another that the danger of bacterial and other decomposition was more reduced, etc. The most commonly accepted standard was that of the nitrogen content. We wished to find a method where as far as possible the process of standardization would be biologic rather than chemical, as representing more nearly what happens in the nonexperimental (allergic) conditions. Complement fixation in pollen treated rabbits had been reported with enough success to seem to justify its use.

Artemisia californica corresponding to ragweed in the East in the frequency with which it caused hay fever was chosen. This was made up in solution by a formula which, on the practicable side, had been the most satisfactory of the many used.

Antigen I	Dried pollen ether extracted forty eight hours	5.65 gm
	in a mixture of	
	Sodium bicarbonate, 1.25%	50.00 cc
	U S P glycerine	50.00 cc

This gave the following analysis:

Nitrogen	0.5 mg per cc
Protein nitrogen	0.25 mg per cc
Protein	1.50 mg cc
Pollen units	50,000 per cc (One P V being 10 ⁶ gm dry, ash free pollen, by definition)

From the Clinic of George Piness, Los Angeles. With acknowledgment of direction from Dr. Hyman Miller and of aid in chemical procedures from Dr. Gordon Allen.
Received for publication May 1, 1936.

TABLE I

RABBIT	TREATMENT			INTERVAL	COMPLEMENT FIXATION
	DATE	DOSAGE	ROUTE		RESULTS
1	10/28/24	2.5 c.c. Antigen II	Intravenously	Tested 4 times in 8 weeks	All negative
2	10/28/24	5.0 c.c. Antigen II	Intraperitoneally	3 weeks	Negative
3	10/28/24	2.5 c.c. Antigen II	Intravenously	5 weeks	Positive
4	11/21/24	Gradual Antigen II	Intravenously	3 weeks	Negative
5	11/21/24	Gradual Antigen II	Intravenously	5 weeks	Positive
7	11/21/24	Gradual Antigen II	Intravenously	3 weeks, after 1st dose	Negative
8	10/30/24	Gradual Antigen II H	Intravenously	1, 2, 4 weeks	Negative
9	10/30/24	Gradual Antigen II H	Intravenously	2 weeks	Negative
12	10/30/24	Gradual Antigen II H	Intravenously	3 weeks	Negative
10	10/30/24	2.5 Antigen II H	Intravenously	3 weeks	Negative
11	10/30/24	2.5 Antigen II H	Intravenously	3 weeks	Negative

Antigen II For inoculation, Antigen I in a dilution of 1 to 5 was made with physiologic salt solution, Mandler filtered, and kept in refrigerator. Further dilution was made at time of using.

Antigen II H Part of the Antigen II was held at 100° for one hour on water-bath, and stored in the refrigerator.

Treatment was of two sorts: gradual, in which the dose began with 50 pollen units and ended with 50,000, given intravenously at two to three day intervals, and massive, in a single dose of 125,000 units given intravenously. Both dosages were borne well, the animals gaining in weight.

At various intervals, complement-fixation tests were made using the standard technique of Kolmer for the Wassermann test, with inactivated serum in a constant amount of 0.1 c.c. Since it was impossible to titrate the antigen for its antigenic value, Antigen II was arbitrarily chosen, being neither hemolytic nor anticomplementary in that amount. (In greater concentration there was a tendency to hemolyze cells, apparently because of the action of the glycerin.) The results are given in Table I. Out of 10 animals, tested with an antigen of about 1 per cent pollen content, only 2 showed complement fixation, and these not to a satisfactory degree. With the incidental use of more concentrated solutions of the pollen, a larger number of positive fixations were secured. These findings seemed to necessitate further experiments.

In the first place, pollen may be considered as weak in antigenic substances, thereby requiring the use of relatively large quantities for a measurable production of antibodies. Along with this the influence of extracives may be in the direction of altering the antigenic quality and of lessening the quantity of active substances. In view of these possibilities the use of untreated pollen in larger doses was undertaken.

In the second place, for the tests the question of a satisfactory antigen was to be determined. Theoretically, this should be made simply and of the lowest dilution not giving anticomplementary action. It would seem that a simple physiologic salt solution would occasion the least changes in the

TABLE II
ANTIGENS USED FOR COMPLEMENT FIXATION TESTS

NO	POLLEN	VARIATION IN PREPARATION	P _H AS USED	POLLEN % AS USED
1	Artem Calif	Repeated nq extraction and concentration by evaporation and in vacuum		2
4	" "	First residue treated with 9%, salt filtrate brought to 0.9%		2
8	Artem Trident.	Mill ground, paper filtered	7.2	2.5
9	" Calif	Mill ground, paper filtered	7.4	1
10	" "	Mill ground, paper filtered	7.4	
12	" "	Mill ground, paper filtered.	7.2	2.5
13	" "	Normal salt extraction	8.2	
14	" "	Mill ground adjusted at once to P _H 7.4, paper filtered.		2.5
15	" "	Mill ground, 10% normal alt frozen 17 times in CO ₂ snow and ether adjusted to P _H 7.4		1
16	" "	Mill ground	7.4	0.5
17	" "	Normal salt solution	7.4	1
18	" "	1% salt solution	7.0	1
19	" "	1.5% NaHCO ₃ , adjusted	7.0	1
20	" "	1% NaCl with equal parts glycerin adjusted	7.0	1
21	" Dracon	1% NaCl, adjusted	7.0	1

TABLE III
COMPLEMENT FIXATION IN SERA RABBIT SERIES 200

DATE	ANTIGEN	VARIATION IN TECHNIQ	RESULTS	PRECEDING TREATMENT
2/28/25	No 1		Negative	3 weeks after first inoculation as above
			Negative	
3/ 2/25	No 6	1½ doses complement sensitized cells	Negative	Normal control
			Serum anticomplementary	Three inoculations
			Serum anticomplementary	Three inoculations
3/ 3/25	No 10 diluted x 2	Fixed 1 hour water bath	xxxx	Two inoculations
			anticomplementary	Normal
			xxxx	Three inoculations
			xxx	Three inoculations
			xx	Two inoculations (stored serum)
3/12/25	Nos 12, 13, 14		xxx	Three inoculations
			anticomplementary	Three inoculations

natural qualities of the substances and that the dilution to be used should be a matter of simple titration

But in the course of making these adjustments a third factor was forced into consideration, and it is here possibly, that the source of the discrepancies in the various reports on pollen fixation may largely lie—that is, in the tendency of rabbit serum to fix complement nonspecifically. This was pointed out particularly by Kolmer,¹ working with lipoidal extracts where inactivated normal rabbit serum gave fixation in about 40 per cent, and with bacterial antigens where the percentage of fixation reached fifty six, with the conclusion that 'this property of rabbit serum of absorbing or fixing complement in a nonspecific manner should be emphasized and better known, for when this animal is used for the purpose of immunization with the object of subsequently conducting complement fixation tests with the serum the

TABLE IV
COMPLEMENT FIXATION IN SERA RABBIT SERIES 115
(HETEROLOGOUS INOCULATIONS)

DATE	ANTIGEN	VARIATION IN TECHNIC	RESULT	PRECEDING TREATMENT
3/ 2/25	6	1½ doses complement, sensitized cells	xxx	One inoculation
3/ 3/25	10, diluted 1:1	1 hour water bath fixation	xxx xxx Negative	" " " " " "
3/ 6/25	49	Results practically the same with both antigens	Negative Anticomplementary x	Normal serum One inoculation " "
3/12/25	12, 13, 14	As above	xxx xxx	" " " "
3/14/25	14	1½ doses complement in presence of known negative serum	Anticomplementary xx Negative	" " " " " "

factor of nonspecific complement fixation enters and may greatly modify the interpretation of results "

Since the chief concern was to secure evidences of antibody rather than to prove, at this point, the value of a given extractive method, the success of Parker² in securing precipitins by inoculating rabbits with whole pollen intraperitoneally prompted the use of this method

Five light young rabbits, Series 200, were given 500 mg of *Artemisia californica* ether-treated, to remove oils and to partially sterilize, suspended in salt solution, and given intraperitoneally. One animal reacted at once with a violent chill, another was found dead after three days. After three weeks the 4 were given 250 mg each. Eight days later one was found dead of pneumonia, without signs of peritonitis. In the mesenteries were found numerous pollen masses of pea and millet sizes which, microscopically, were found to consist of amorphous material and many intact pollen granules. This method would then correspond because of the slow absorption to that of the graduated dosage type. A third rabbit was very emaciated after two weeks and was bled to death. The remaining two survived a third dose of 250 mg. Another group of 5 grey rabbits, Series 115, bore without ill effects a treatment with 250 mg, and 5 gained weight under the graduated intravenous dosage of 2 per cent extract of pollen in normal salt and sodium bicarbonate as used by Parker for precipitin tests. This extract was used also as antigen in the complement-fixation tests, which followed the standard technic of Kolmer, except that perforce, the titration for antigenic value was omitted. The antigens, freshly prepared, were passed through hard filter paper rather than through the Berkefeld filter in order to conserve the protein content. A practically clear solution resulted if the suspension was first centrifuged at high speed. The preparation of the various lots of antigen are listed in Table II.

Tables III, IV and V give the results of 36 tests of sera from treated animals. Seventeen were positive, 6 were negative or doubtful and 7 anti-complementary. But at the same time, 6 normal sera gave 2 positive and

TABLE V
COMPLEMENT FIXATION IN SERA RABBIT SERIES 125

DATE	ANTIOEN	VARIATION IN TECHINIO	RESULTS	PRECEDING TREATMENT
3/6/25	4, 8, 9		xxxx xxxx 2 anticomplementary 4 8 9	Seven doses " "
3/12/25	12, 13, 14		0 xxx x xx Negative with 14 Anticomplementary 12 13 14	" " " " Normal Seven doses " "
3/14/25	14	14 doses complement in presence of known negative serum	xxx xxx xx xx xxx xx xxxx Negative	Normal " "

TABLE VI
COMPLEMENT FIXATION IN RABBIT SERUM*

NORMAL SERUM	ANTIGEN			NO ANTIGEN
	15	16	17	
0.12 c.c.	xxxx	xxx	xxxx	0
0.18 c.c.	xxxx	xx	xxx	0
0.24 c.c.	xx	x	xx	0
IMMUNE SERUM				
0.12 c.c.	xxxx	xxx	xxxx	0
0.18 c.c.	xxx	xx	xxx	0
0.24 c.c.	xxx	0	0	0

*In each case 0.12 c.c. serum contains a double hemolytic unit and the results are in degree of fixation.

1 anticomplementary reaction. These very irregular results, including fixation with a heterologous antigen, *A. tridentata* suggested the use of a technique allowing the use of unheated serum, thereby reducing the tendency to non specific fixation,³ and conserving the antibody.

In studying complement fixation in human sera, using streptococcus in suspension as antigen, Burbank and Hadjopoulos⁴ secured satisfactory results with a system adapted to meet the difficulties inherent in the inadequacy of a weak antigen and in the deleterious effect of heat on antibody in process of inactivation.

The active serum was titrated for its complement content in the presence of 0.5 c.c. of 0.5 per cent sensitized sheep cells. The test set up was two series of four tubes each, in which increasing complementary units of sera were pipetted. To one series was added 0.5 c.c. of the antigen of a dilution twice that of the anticomplementary unit determined in the presence of pooled negative sera. As prepared, the antigens were not found to be hemolytic in double the dose used, either before or after adjustment, but in dilutions lower than the anticomplementary values, there was a tendency to decolorize without clearing—as with saponin—inhibited by adjustment toward a P_{H} of 7.0. After one hour's fixation in the water bath, the sensitized sheep cells were added. A typical reaction is recorded in Table VI.

With a hemolytic system so adjusted that the unit of complement fell around 0.1 c.c. serum, six sera, read as positive under the Kolmer system, gave with antigens 15, 16, 17 no more fixation than the pooled sera of three normal rabbits, using the complement of each serum, determined before and during the test. The work was checked, with the same results, by using pooled sera of both immunized and normal animals to obviate the excessive manipulation incident to the self-complement titration, using antigens 18-21. From this one could only say that the antibody titer and the anticomplementary titer lay too close together to make demonstration of antibody content of value in this case.

DISCUSSION

Where the immunizing agent and the test antigen are used in states as little changed as possible and as nearly alike as possible, failure to secure satisfactory immune body reactions (errors of technique not considered) must be due either to the known poverty of pollen in antigenic substances, or to an actual deficiency in antibody content. On the other hand, where reactions are secured, they must be discounted by the tendency of rabbit serum to fix nonspecifically unless this condition is suitably controlled. From the experiments here reported, it would seem that both factors may be responsible for the variable results reported for complement fixation in pollen-treated animals and that the test is inadequate as a means of standardizing pollen extracts.

REFERENCES

- ¹Kolmer Jour Infect Dis, 1916, viii, 20
- ²Parker Jour Immunol, 1924, iv, 6
- ³Kolmer Jour Infect Dis, 1916, viii, 64
- ⁴Burbank and Hadjopoulos Jour Am Med Assn, Feb 28, 1925, lxxiv, 637

THE ANTICOMPLEMENTARY REACTION OF BLOOD SERUM TO THE KOLMER COMPLEMENT FIXATION TEST CONTROLLED BY THE REACTION TO THE KAHN PRECIPITATION TEST*

By S WILLIAM BECKER,† M D, ROCHESTER MINN

A STUDY on the anticomplementary reaction to the Kolmer complement fixation test on both blood serum and cerebrospinal fluid was presented by Sanford in May, 1925. All the samples of cerebrospinal fluid on which this reaction was obtained were from patients almost definitely proved to have syphilis, and 59 per cent of the samples of blood serum were from syphilitic patients. As a continuation of this study I have performed the Kahn precipitation test on 112 samples of blood serum which have yielded anticomplementary reactions to the Kolmer test. These samples were obtained from sixty nine patients seen at the Mayo Clinic.

Kolmer's standardized quantitative complement fixation test was employed, except that only the first second and control tubes were used and in each case the serum was inactivated for twenty minutes instead of fifteen. The degree of reaction in the three tubes was recorded in all but ten instances. In the latter the degree of reaction in only the control tube was known this was designated as "strongly anticomplementary," in two instances and as "weakly anticomplementary," in eight instances. The Kahn precipitation tests were performed immediately after the Kolmer tests about twenty four hours after withdrawal of the blood. The serum had been left on the clot at room temperature. The method of Kahn was followed with a slight modification the tubes being shaken vigorously by hand instead of being shaken by machine. For convenience the results 3+ and 4+ are designated "strongly positive" and the results 1+ and 2+ "weakly positive" (Tables I II III IV, V VI,

TABLE I

STRONGLY POSITIVE REACTION TO KAHN TEST WITH DEFINITE HISTORY OR SIGNS OF SYPHILIS

RESULTS OF KOLMER TESTS	CASES
+++ on all examinations (three to six)-----	3
+++ on one to three examinations with additional positive reactions-----	10
++ in first two tubes and 1 to 4 in control tube on different examinations, with additional positive reactions in all but one instance-----	11
Less than ++ in first two tubes and 1 or 2 in control tube with additional positive reactions-----	3

TABLE II

STRONGLY POSITIVE REACTION TO KAHN TEST WITHOUT DEFINITE HISTORY OR SIGNS OF SYPHILIS

RESULTS OF KOLMER TESTS	CASES
+++ on one examination (Case 1)-----	1
+++ but negative on same serum twenty four hours later (Case 2)-----	1
+++ on one examination (Case 3)-----	1

Submitted for publication May 1 1926
†Fellow in Dermatology The Mayo Foundation Rochester Minnesota

CASE 7—A woman, aged forty four, complained of "liver trouble" of six months' duration. At operation she was found to have chronic cholecystitis and biliary cirrhosis. There was no history of syphilis. The reaction to the Kolmer test on the blood was negative. The Kahn test was not performed on this serum. On account of enlargement of the left lobe of the liver, the patient was given a provocative injection of arsphenamine. The results of the five daily Kolmer tests were negative, weakly anticomplementary, and negative three times. The reaction to the Kahn tests were weakly positive twice and negative three times. The spinal fluid was normal. The husband's reactions to the Kolmer and Kahn tests were negative, and his cerebrospinal fluid was normal. The diagnosis was indeterminate as regards syphilis.

Many of the patients with positive Kolmer reactions, never stronger than 33, were investigated by the multiple-procedure diagnostic attack of Stokes, but in no instance could definite signs of syphilis be elicited. The difficulty in evaluating the weakly positive and moderately positive Kolmer reaction in the absence of history and evidence of syphilis will be discussed elsewhere.¹

Six of the patients with general paresis (Table VII) and the tabetic patient with gastric crisis had just completed a course of malarial treatment. Each patient had had positive Kolmer reactions previously, but never an anticomplementary reaction. The absence of primary and secondary syphilis from the list may be of significance, or it may be attributable to the paucity from the list may be of significance (Table VIII).

COMMENT

If the cases in Table II are included in the syphilitic, and those in Table IV in the nonsyphilitic cases, there are thirty-three (48 per cent) syphilitic and thirty-six (52 per cent) nonsyphilitic cases. If the cases in which "artificial" anticomplementary reactions were obtained on the serum (the cases in which malarial treatment was being given for neurosyphilis) are disregarded there are twenty-six (42 per cent) syphilitic and thirty-six (58 per cent) nonsyphilitic cases. This percentage is somewhat less than that of the syphilitic cases in Sanford's series (59 per cent), but both series are too small to permit of final conclusions.

There was one instance of anticomplementary reaction on both blood and cerebrospinal fluid, a combination not present in Sanford's series.

Three-quarters of the nonsyphilitic patients were suffering from infectious disease. This, coupled with the fact that anticomplementary reactions were produced in a certain percentage of neurosyphilitic patients by inoculation with malaria, suggests infection as a cause for the anticomplementary reaction.

My results are in accord with those of Kolmer, who says, "If the serum is very slightly anticomplementary and the front tube shows complete inhibition of hemolysis, the reaction is in all probability positive. If the rear tube, however, shows marked inhibition of hemolysis, indicating that it is highly anticomplementary, the result cannot be determined, but a retest with fresh serum must be made."

SUMMARY

Of a series of sixty nine patients whose blood serums were anticomplementary on 112 occasions, 48 per cent were found to be syphilitic. More than three fourths of the nonsyphilitic patients were suffering from infectious disease. There is no evidence that the substance which fixes complement without antigen also produces a positive precipitation reaction. Avoidance of the anticomplementary Kolmer reactions by substituting the Kahn test does not permit of satisfactory evaluation in all cases. However, the Kahn precipitation test is a valuable addition to the multiple procedure diagnostic attack of Stokes in the study of patients whose serum gives an anticomplementary reaction by the Kolmer complement fixation test.

REFERENCES

- Hecker S W. Unpublished data.
 Crug, C F, and Nichols H J. The Effect of the Ingestion of Alcohol on the Results of the Complement Fixation Test in Syphilis. *Jour Am Med Assn* 1911 *lxii* 474-476.
 Kahn, H L. The Kahn Precipitation Test for Syphilis. *Am Jour Pub Health* 1924, *xii*, 498-507.
 Kolmer, J A. A Practical Textbook of Infection, Immunity and Biologic Therapy ed 3 Philadelphia Saunders, 1924, pp 472-488.
 O'Leary I A. The Provocative Procedures in the Diagnosis of Syphilis, *Arch Dermat and Syph* 1920, *ii*, 348-353.
 Sanford, A H. The Anticomplementary Reaction of Blood Serum or of Spinal Fluid. *Journ. Lab and Clin Med*, 1920, *xi* 413-420.
 Stokes J H. The Clinical Approach to Syphilis with Suggestions for its Revival and Development. *Arch Dermat and Syph*, 1920 *ii* 473-49.

COMPARISON BETWEEN THE KAHN FLOCCULATION TEST THE KOLMER WASSERMANN TEST AND THE RUEDIGER WASSERMANN TEST*

By E H RUEDIGER M.D., HOLLYWOOD CALIF

IN A previous report I¹ showed that my modification of the Wassermann test is more sensitive than the Kolmer modification of the Wassermann test. During the last year the Kahn flocculation test has attracted a great deal of attention and many reports are appearing in the medical journals. I shall briefly refer to some of these reports. Levin made parallel Kahn and Wassermann tests on 2542 serums with 92.2 per cent agreement in the results. Boas² reports 91 per cent agreement with the Kahn and Wassermann tests. Owen and Cope³ prefer the Wassermann test to the Kahn test. Redfield⁴ prefers the Kahn test because of its simplicity. Houghton⁵ came to the conclusion that the Kahn test possesses superior qualities over the Wassermann test and other precipitation tests now in use because it is much more simple and less time consuming. The United States Navy⁶ adopted the Kahn test as the official serologic test for syphilis and yaws. In a later report Owen and Cope⁸ show 93.8 per cent agreement with the Kahn test and the Kolmer Wassermann test. Occasionally they had a positive result with the Kolmer Wassermann test and a negative result with the Kahn test and a

From the Hollywood Clinical Laboratory, Hollywood, California.
 Received for publication May 1, 1926.

TABLE I

COMPARISON OF THE KAHN FLOCCULATION TEST, THE KOLMER WASSERMANN TEST, AND THE RUEDIGER WASSERMANN TEST

NO OF SPECIMEN	KAHN TEST	KOLMER WASSERMANN	RUEDIGER WASSERMANN	REMARKS
			Units per c c	
1	Negative	Negative	20	Syphilitic history
2	+++	44400	250	Syphilis
3	+++	44400	200	Syphilis
4	++++	44400	200	Syphilis
5	++++	44420	350	Syphilis
6	±	40000	36	Treated syphilis
7	Negative	Negative	20	Husband syphilitic
8	+	20000	36	Syphilis
9	++	43000	50	Syphilis
10	+++	44300	180	Syphilis
11	+	40000	100	Syphilis
12	++++	44430	500	Syphilis
13	Negative	Negative	6	Treated syphilis
14	+	21000	50	Syphilis
15	+++	44420	300	Syphilis
16	+++	44300	150	Husband of No 10
17	Negative	Negative	40	Treated child of No 7
18	++++	44400	350	Syphilis
19	Negative	Negative	20	Treated syphilis
20	Negative	Negative	6	Early chancre
21	++++	44410	200	No 20, 11 days later
22	+++	44300	150	Syphilis
23	++++	44420	400	Syphilis
24	++++	44444	1500	Secondary syphilis
25	++++	44410	500	Syphilis
26	+	20000	36	Syphilis
27	++++	44400	300	Syphilis
28	Negative	Negative	15	Treated syphilis
29	+++	44400	250	Syphilis
30	+	33200	200	Treated syphilis
31	Negative	Negative	100	Treated syphilis
32	++	44400	200	No 21, a month later
33	++++	44400	150	Syphilis
34	++	44000	75	Syphilis
35	++++	44420	400	Syphilis
36	++	40000	60	Syphilis
37	Negative	Negative	12	Treated syphilis
38	Negative	Negative	20	Treated syphilis
39	+++	44000	100	Syphilis
40	Negative	Negative	20	Treated syphilis
41	++	44000	36	Treated syphilis
42	+	10000	20	Treated syphilis
43	+++	44400	100	Syphilis
44	++	44200	36	Syphilis
45	Negative	Negative	12	Syphilis, stomach trouble
46	+++	44430	50	Secondary syphilis
47	Negative	Negative	12	Treated syphilis
48	++++	44420	100	Syphilis
49	Negative	Negative	6	Wife of No 50
50	+++	44400	100	Secondary syphilis
51	+++	44440	300	Syphilis
52	Negative	44000	50	Treated syphilis
53	+	44000	36	Syphilis
54	Negative	Negative	12	Treated syphilis
55	+	44000	36	Syphilis
56	+++	44400	60	Chancre 3 weeks old
57	Negative	Negative	20	Treated syphilis
58	+++	44400	60	No 56, 3 days later
59	++	34300	30	Treated syphilis
60	+++	44400	70	Syphilis

TABLE I—CONT'D

NO OF SPECIMEN	KAHN TEST	KOLMER WASSERMANN	RUEDIGER WASSERMANN	REMARKS
			Units per c c	
61	+	Negative	15	No 49, 17 days later
62	+++	44400	70	Syphilis
63	+	11000	21	Treated syphilis
64	Negative	Negative	12	Treated syphilis
65	+++	44420	200	Chancre
66	++++	44430	300	Wife of No 65
67	Negative	31000	36	Treated syphilis
68	Negative	32000	36	Chancre 3 (1) days old
69	Negative	Negative	12	Chancre 6 days old
70	Negative	Negative	20	No 69, 2 days later
71	Negative	Negative	4	Spinal fluid, syphilis
72	++	44100	50	No 70 5 days later
73	+++	44400	150	Syphilis
74	Negative	Negative	12	Treated syphilis
75	Negative	Negative	12	Treated syphilis
76	++	44100	36	Treated syphilis
77	+	44200	36	Syphilis
78	++++	44443	350	Syphilis
79	+++	44410	200	Syphilis
80	+++	44430	200	Syphilis
81	+++	44400	50	Syphilis
82	++++	44440	400	Syphilis
83	+	44400	70	Syphilis
84	++++	44441	400	Syphilis
85	+	22000	36	Syphilis
86	+	11000	0	Spinal fluid, syphilis
87	+	01000	6	Spinal fluid syphilis
88	+	32000	10	Syphilis
89	+	Negative	30	Syphilis
90	+++	44400	200	Syphilis
91	+++	44400	200	Syphilis
92	Negative	Negative	4	Syphilis
93	++	12000	36	Treated syphilis
94	Negative	23200	36	Treated syphilis
95	Negative	Negative	12	Treated syphilis
96	+	Negative	12	Treated syphilis
97	++++	44440	300	Syphilis
98	Negative	44200	24	Treated syphilis
99	+	22000	6	Treated syphilis
100	Negative	Negative	12	Treated syphilis

few cases gave positive results with the Kahn test and negative results with the Kolmer Wassermann test Giordano⁹ made parallel Kahn and Kolmer Wassermann tests on 2,540 serums and the results agreed in 96 per cent of the cases Occasionally one test gave a positive result and the other test gave a negative result In a clinical study of 110 cases Kelly¹⁰ ran the Kahn flocculation test and the Kolmer Wassermann test on the same serums In 95.45 per cent of the tests the results agreed The serums of three persons who were known to be syphilitic gave positive results with the Kahn test and negative results with the Kolmer Wassermann test and the serum of one person gave a negative result with the Kahn test and a positive result with the Kolmer Wassermann test

METHODS

The Kahn test was done as described by Kahn and with antigen kindly supplied by Kahn In the Kolmer Wassermann test I used the human hemolytic system, otherwise I followed Kolmer's directions The Ruediger Was

seimann test was done in accordance with the descriptions previously given,^{1 11 12, 13} except for the following modification To 100 cc of the antigen 50 mg of cholesterol was added, the complement was always diluted 1 10, and 15 unit of hemolytic amboceptor was used

My present report deals with the results I obtained with the Kahn flocculation test, the Kolmer-Wassermann test, and my own modification of the Wassermann test on 260 consecutive specimens Of the 260 specimens, 160 gave negative results with all three methods and 100 specimens gave positive results with one or more methods Among those that gave negative results by all three methods were at least two bloods that came from persons who clinically were syphilitic One patient had two almost typical Charcot joints and the other was diagnosed as syphilitic marasmus with arteriosclerosis Both patients were given antisyphilitic treatment and marked improvement followed The spinal fluids were not examined

The more important part of this report deals with the specimens that gave positive results, and are given in detail in Table I

The accompanying table shows the results that were obtained with the serums that were positive by one or more methods Those which gave positive results need no further discussion but some explanation is given on those serums in which the results disagree

Serum No 1 came from a young married woman in her first pregnancy who was admitted to a hospital for some minor trouble I was studying the Wassermann test in pregnancy at the time, and therefore included her The Kahn test gave a negative result, the Kolmer-Wassermann test gave a negative result and my method showed 20 fixing units per cubic centimeter of serum There were no other signs of syphilis, the positive Ruediger-Wassermann test was ignored and the patient was sent home About six weeks later her family physician sent me a specimen of her blood for Wassermann test and advised me that she had a miscarriage and that he obtained a syphilitic history The serum was retested and the results were identical with the first

Serum No 7 was obtained from a married woman who has two living syphilitic children, and a tabetic husband, and has had several miscarriages Repeated Wassermann tests done at other laboratories gave negative results, while I got negative results with the Kahn and Kolmer tests and 20 units fixation with my method In early primary syphilis my method always gave positive results before positive results were obtained with the Kahn test or with the Kolmer test as is shown by Serums Nos 20, 49, 68, 69 and 70 Under treatment the Kahn test and the Kolmer test became negative much sooner than my method, Serums Nos 13, 17, 19, 28, 31, 37, 38, 40, 45, 47, 52, 54, 57, 64, 67, 74, 75, 89, 92, 94, 95, 96, 98 and 100 show these results With spinal fluid from syphilitics my method gave positive results when the Kahn test and the Kolmer test gave negative results as is shown by No 71 Serum No 45 came from a patient who had severe stomach trouble There was a history of syphilis contracted more than twenty years before The Kahn test and the Kolmer test gave negative results and with my method I demonstrated 12 fixing units per cubic centimeter of serum The patient was given

antisyphilitic treatment and he improved rapidly. In a previous attack of apparently the same nature a gastroenterostomy was done, probably because the Wassermann test gave a negative result.

SUMMARY

Parallel Kahn flocculation tests, Kolmer Wassermann tests and Ruediger Wassermann tests were done on 260 consecutive specimens.

Of these 260 specimens 160 gave negative results by all three methods and 100 gave positive results by one or more methods. Among the 160 specimens that gave negative results by all three methods were at least two which came from patients who were clinically considered chronic syphilitics. They were treated as syphilitics and improved greatly. The blood serum only was tested.

All of the 100 specimens that gave positive results by one or more methods came from syphilitics and all gave positive results by the Ruediger Wassermann test, 70 gave positive results by the Kahn test, and 72 gave positive results by the Kolmer test. With three specimens the Kahn test gave positive results while the Kolmer test gave negative results and with five other specimens the Kolmer test gave positive results and the Kahn test gave negative results. There was 70 per cent agreement between the Kahn flocculation test and the Ruediger Wassermann test and 72 per cent agreement between the Kolmer Wassermann test and the Ruediger Wassermann test. Of the specimens that gave positive results by the Ruediger Wassermann test and negative results by the Kahn flocculation test and the Kolmer Wassermann test, four were blood serums from patients with primary syphilis, two were blood serums from more or less chronic syphilitics that had always given negative results with the Wassermann test, one was a spinal fluid from a syphilitic, and the others were blood serums from treated syphilitics.

REFERENCES

- ¹Ruediger, E. H. Plea in Favor of Standardized Wassermann Test. *California and West Med* 1924, xxii 548-553.
- ²Levin, W. Kahn Precipitation Test as Routine Test in Diagnosis and Treatment of Syphilis. *Northwest Med*, 1924, xxiii 543-547.
- ³Boas, H. Kahn's Precipitation Test. *Dermat Ztschr* 1925, xliii 140-148.
- ⁴Owen, R. G. and Cope, H. E. Kahn Precipitation Test for Syphilis. *Jour Michigan Med Soc* 1925, xiv, 94-98.
- ⁵Redfield, K. T. Kahn Test as Aid in Diagnosis of Syphilis. *Am Jour Syph* 1925, ix 354-359.
- ⁶Houghton, J. E. Expediting Diagnosis of Syphilis by Employment of Kahn Precipitation Test. *U. S. Nav Med Bull* 1925, xxiii 547-53.
- ⁷Navy Adopts Kahn Test as Standard. *Jour Am Med Assn* 1926, lxxvi 210.
- ⁸Owen, R. G. and Cope, H. E. Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test. *Jour Lab and Clin Med* 1926, xi 432-434.
- ⁹Giordano, A. S. Kahn Precipitation Test as Compared with Kolmer Complement Fixation Test. *Jour Lab and Clin Med* 1926, xi 435-437.
- ¹⁰Kelly, R. L. Clinical Study of Kahn Precipitation Test and Kolmer Complement Fixation Test. *Jour Lab and Clin Med* 1926, xi 437-445.
- ¹¹Ruediger, E. H. Discrepancies in the Wassermann Reaction. *Journal Lancet* Oct 15 1920.
- ¹²Ruediger, E. H. Proposed Standard Technique for the Wassermann Test. *Ann Clin Med* 1924, ii 237.
- ¹³Ruediger, E. H. Quantitative Wassermann Test. *Jour Lab and Clin Med*, 1925, x 303-310.

THE RÔLE OF THE WASHINGS OF STREPTOCOCCUS SCARLATINAE IN SCARLET FEVER *

BY N S FERRY, PH B, M D, AND L W FISHER, B S, DETROIT, MICH

FOR carrying out the cutaneous reaction, referred to as the Dick reaction, for determining susceptibility to the *Streptococcus scarlatinae* and, presumably to scarlet fever, and also for prophylactic inoculation against the disease, the Dick toxin, as originally described by its sponsors¹ was prepared by passing the water of condensation of a sheep's blood agar growth of the scarlet fever streptococcus through a Berkefeld V filter. The filtered culture containing the soluble toxin from the organisms growing in the condensation fluid was diluted according to its requirements.

Later the Dicks, and also Zingher,² after the method of Williams, Hussey, and Banzhaf,³ in New York, used a toxin prepared from a broth culture—the Dicks by the addition of sheep blood to the media, while Zingher advocated the addition of horse blood for enrichment purposes—the cultures being incubated, at the usual temperature, from three to six days. The toxin has also been obtained in plain broth by Kukbride and Wheeler⁴ and in 0.2 per cent glucose broth by ourselves—in fact it has been described by several investigators as having been obtained in almost any medium in which the *Streptococcus scarlatinae* will develop. The toxin, therefore, while not noticeably toxic to animals in the strengths obtained at present, has been described as an extracellular toxin from its action on the human.

The authors, following a method first reported by them in June, 1924,⁵ whereby the washings of various organisms were successfully used as antigens for immunizing purpose, prepared an antigen in like manner from the washings of the *Streptococcus scarlatinae*, which not only produced the cutaneous susceptibility reaction in susceptible individuals but stimulated an active immunity against the toxin as well. The results with these washings of the *Streptococcus scarlatinae* corresponded very favorably with those produced with scarlet fever toxin, prepared in the usual manner from broth cultures, obtained from the Hygienic Laboratory, Washington. This shows that the same antigenic substance was present in both the washings and the toxin.

The work on the washings of the *Streptococcus scarlatinae* was reported⁶ at the meeting of the Association of Immunologists in Washington, April, 1924, as follows: "Recently with washings prepared from a streptococcus isolated from scarlet fever we have been able to produce a positive skin reaction in individuals susceptible to scarlet fever, which reaction would become negative if the washings were mixed with convalescent scarlet fever serum, similar to the results obtained by others with the Dick toxin." A detailed account of this experimental work is given at this time.

*From the Medical Research Laboratories Parke Davis and Company Detroit.
Received for publication May 23 1926

When this type of antigen is prepared from bacterial washings the organisms are incubated for twenty four hours on solid media and washed off with salt solution, either with or without preservative. The suspension of organisms, which usually contains an unappreciable amount of water of condensation, is mechanically shaken for a few minutes and immediately passed through a Sharples centrifuge leaving a clear watery solution containing a very small percentage of bacterial proteins. To this type of antigen the name ecto antigen was given, as it was clearly not of the extracellular type nor was it of the endocellular type, and to distinguish antigens prepared in this way from others and for clinical purposes the name immunogen was used.

As washings from such organisms as the pneumococcus typhoid bacillus, pertussis bacillus gonococcus and streptococci other than the Streptococcus scarlatinae gave evidence of antigenic properties of extremely high immunizing value it was thought probable that the washings of the scarlet fever streptococcus would behave in a similar manner. With that thought in mind preliminary tests were carried out on members of our laboratory staff giving negative histories to scarlet fever with favorable results.

Later tests were carried on at three other institutions in Detroit. The Protestant Orphan Asylum, St Vincent's Orphan Asylum and St Francis Home for Orphan Boys.

At the first institution all of the children had previously been given the cutaneous test with the regular toxin and the susceptible ones given three immunizing doses of the toxin. Retests of these positive cases two months after the last immunizing with the antigen prepared by washing the Streptococcus scarlatinae, controlled by the Government standard toxin, showed that 80 per cent had remained successfully immunized for two months.

At St Vincent's Orphan Asylum an epidemic of scarlet fever was in progress at the time the work was instituted so that only those were tested who were considered normal and were not convalescing from the disease. Of these there were 195 and all were skin tested with the washings on one arm and controlled with the standard toxin on the other. Of the 44 giving positive reactions half were immunized with three doses of the regular toxin and half with three doses of the washings, and all were retested two months after with the washings controlled as previously with the standard toxin. The first dose of the toxin and also of the washings contained an equivalent of 250 skin test doses of toxin per cubic centimeter the second dose contained 500 skin test doses and the third 1000 skin test doses. All injections were given at intervals of one week. While rather disagreeable general reactions were recorded in three cases of the older girls following the injections of the toxin and also several severely swollen arms were noted none of these symptoms were evidenced in the cases where the washings were used. On retest two months after the last immunizing dose 85 per cent of the cases gave negative reactions to the intra-cutaneous injection of the material showing that 15 per cent were still susceptible to the disease if the test is as reliable as it is thought to be.

It is interesting to note that this work was undertaken during an epidemic of scarlet fever at St Vincent's Asylum and not another case was

reported after the prophylactic injections were started. Whether the prophylactic injections had anything to do with the abating of the epidemic, we are not able to state. It would seem plausible, however, and because of the fact that half of the remaining children were immunized with toxin prepared in the regular way and half with the washings, it is only fair to assume that each product had an equal share in the successful termination of the epidemic.

At the St. Francis Home for Boys, all of the boys, 530 in number, were given the cutaneous test with washings, controlled with the standard toxin, and 92 were found to be positive reactors. These were all immunized with the washings, a dosage being employed much weaker than that used in the previous experiment at St. Vincent's Asylum, and about half the total number of skin tests being used. Even with this much smaller amount of antigen being used, it was found that 57.7 per cent of the susceptibles were successfully immunized, they were retested two months after the last immunizing dose.

Since this work with washings of the *Streptococcus scarlatinae* was first reported, it has been corroborated by Henry and Lewis,⁷ in so far as the preparation of the antigen is concerned, in a report to the Medical Research Council of England. They used a similar method of preparation for their toxin, and then further precipitated the antigen with various volumes of alcohol and desiccated it. Favorable results were reported by them with dried material diluted to original volume with salt solution, when this material was used for the skin test. Prophylactic inoculations were not undertaken by these authors.

DISCUSSION

That the *Streptococcus scarlatinae* produces a soluble extracellular antigen, toxic in nature for the human, there is no doubt. Ample proof to substantiate this is at hand, especially in the production of the rash after large doses of this antigen given subcutaneously and, also, in the production of an antiserum, with this antigen which will neutralize scarlet fever toxin.

That this antigen is also of the nature of an ectoantigen has been shown by the above experiments with the washings.

It is very evident, therefore, that this antigen differs in many respects from that produced by the diphtheria and tetanus bacilli, as it has never been shown that washings from twenty-four-hour growths of these organisms on solid media produce antigens very toxic in nature, or that the toxin from the *Streptococcus scarlatinae* is very toxic for animals even in large doses.

CONCLUSIONS

Washings of the *Streptococcus scarlatinae* grown on solid media contain an antigen which, when injected intracutaneously into the human, will produce the cutaneous susceptibility test called the Dick test, similar to the broth filtrate.

These washings will also produce a prophylactic immunity against the scarlet fever toxin similar to that produced by the toxin itself.

The specific antigen of the streptococcus seems to be both extracellular and ectoantigenic in nature.

REFERENCES

- ¹Dick and Dick Jour Am Med Assn Oct 6 1923 LXXXI 1116
²Zingher Jour Am Med Assn, Aug 9, 1924 LXXXII 432
³Williams Hussey and Banzhaf Proc Soc Exper Biol and Med March 1924, XXI 291
⁴Reported at the Meeting of the Am Assn of Immunologists Washington April 1924
⁵Ferry and Fisher Brit Jour Exper Path June and August, 1924 v, 185 and 205
⁶Ferry and Fisher JOUR LAB AND CLIN MED July 1925, v, 817
⁷Henry and Lewis Lancet April 4 1925 i 712
-

Specialist in Pathology Needed at Knoxville, Iowa, Hospital

The United States Civil Service Commission states that there is a vacancy in a position of specialist in pathology at the Veterans' Bureau Hospital at Knoxville Iowa, and that applications are being received for the position.

The entrance salary is \$3 800 a year. Promotion to higher grades may be made in accordance with the civil service rules.

Full information and application blanks may be obtained from the United States Civil Service Commission Washington D C

LABORATORY METHODS

A COMPARISON OF THE FOLIN-WU AND NEW BENEDICT METHODS FOR THE ESTIMATION OF BLOOD SUGAR *CM*

By ARNOLD E. OSTERBERG, PH D., AND JOY STRUNK, B A., ROCHESTER, MINN *

THE Folin-Wu procedure for the estimation of blood sugar has been adopted as a routine method in many laboratories. As a result physicians have come to regard 100 mg of glucose for each 100 cc of blood, in a laboratory report, as approximately the normal value.

It is not theoretically correct to assume that all of the reduction obtained when the Folin-Wu copper reagent is added to a protein-free blood filtrate is due to glucose, since it is well known that when this reagent is applied to normal urine, values are obtained for the glucose present which are two or three times that known to be correct. In spite of this, however, the method has served admirably for the routine procedure in the clinical laboratory.

Since the recent publication by Benedict of a modified Folin-Wu copper reagent it was interesting to determine whether it was worth while, from a clinical standpoint, to alter the normal values for blood sugar and to change the procedure for the estimation of blood sugar from that of Folin-Wu to Benedict's newer procedure†. Benedict says that his new solution when applied directly to urine gives only one-tenth of the error of the Folin-Wu procedure, and that the results obtained are comparable when applied to urine that has been treated with Lloyd's reagent. The principal difference which Benedict has initiated in his modified solution is that the copper reagent has been varied so that the amount of citrate in the original solution has been increased, whereas the concentration of copper and carbonate has been decreased. Also, a small amount of sodium bisulphite has been added to increase the amount of cuprous oxide produced by a given amount of sugar. For the development of the color of the cuprous oxide, Benedict's uric acid reagent is used, to which has been added 5 per cent formaldehyde to prevent any increase of color by the sodium bisulphite. By this method Benedict has obtained as normal values for blood sugar, an average of 75 mg for each 100 cc of blood, and he believes that even this figure may be too high. He would place the normal value at approximately 60 mg for each 100 cc of blood. Folin's figures were not so low with the new method. In fact, he says that if care is taken to insure the use of fresh solutions and to prevent the oxidation of the sodium bisulphite by atmospheric oxygen, results are

*From Section on Clinical Chemistry, Mayo Clinic, Rochester, Minnesota.

Submitted for publication July 31, 1926.

Received for publication August 2, 1926.

†Benedict has in a more recent paper again changed the method. This article however concerns itself only with the modifications first described.

obtained which are practically the same as those given by the Folin Wu procedure

In order to compare the two methods we determined simultaneously the glucose content of four blood filtrates by the two procedures. Pure glucose was then added to the filtrate in varying amounts and the total glucose content redetermined (Table I)

In general the error of recovery by Benedict's new method is less than by the Folin Wu procedure. It would seem from these data that the amount of reduction obtained from a given amount of glucose is more nearly quantitative by the Benedict procedure. None of the errors by the Benedict procedure are of particular clinical significance since the greater ones are present only in those filtrates with high sugar values.

As a comparison of the two procedures in the routine estimation of blood sugar for clinical purposes we have taken as a basis for our opinion glucose estimations made simultaneously on the same blood filtrates by the two methods. The results were obtained by the analysis of 194 consecutive blood specimens taken in the laboratory with no regard to clinical diagnosis (Table II)

TABLE I
COMPARISON OF RECOVERY OF GLUCOSE ADDED TO BLOOD FILTRATES

SAMPLE	FILTRATE AND GLUCOSE, MG FOR EACH 100 CC	MG OF GLUCOSE FOR EACH 100 CC OF BLOOD		CALCULATED NUMBER OF MG OF GLUCOSE FOR EACH 100 CC OF BLOOD		ERROR IN MG OF GLUCOSE FOR EACH 100 CC OF BLOOD	
		FOLIN WU	BENEDICT	FOLIN WU	BENEDICT	FOLIN WU	BENEDICT
A		93	80				
A	20	112	95	113	100	- 1	- 5
A	60	162	136	153	140	+ 0	- 4
A	100	200	184	193	180	+13	+ 4
A	140	250	224	233	220	+17	+ 4
A	180	302	266	273	260	+20	+ 0
A	220	352	306	313	300	+30	+ 0
A	260	408	356	353	340	+33	+16
B		93	80				
B	20	113	100	113	100	0	0
B	60	162	138	153	140	+ 9	- 2
B	100	188	178	193	180	- 5	- 2
B	140	230	224	233	220	- 3	+ 4
B	180	276	258	273	260	+ 3	- 2
B	220	326	300	313	300	+13	0
B	260	362	332	353	340	+ 9	- 8
C		95	83				
C	20	119	103	115	103	+ 4	0
C	60	158	150	155	143	+ 3	+ 7
C	100	198	184	195	183	+ 3	+ 1
C	140	246	216	235	223	+11	- 7
C	180	302	266	275	263	+26	+ 3
C	220	346	310	315	303	+31	+ 7
C	260	396	350	355	343	+41	+ 7
D		97	90				
D	20	121	108	117	110	+ 4	- 2
D	60	166	153	157	150	+ 9	+ 3
D	100	192	198	197	190	- 5	+ 8
D	140	242	238	237	230	+ 5	+ 8
D	180	284	278	277	270	+ 7	+ 8
D	220	324	322	317	310	+ 7	+12
D	260	380	380	357	350	+23	+20

TABLE II

THE GLUCOSE CONTENT OF BLOOD ESTIMATED BY THE FOLIN WU AND BENEDICT'S NEW PROCEDURE

NUMBER	GM OF GLUCOSE FOR EACH 100 CC OF BLOOD			NUMBER	GM OF GLUCOSE FOR EACH 100 CC OF BLOOD		
	FOLIN WU	BENEDICT	DIFFERENCE		FOLIN-WU	BENEDICT	DIFFERENCE
1	073	069	004	61	100	098	002
2	083	077	006	62	101	090	011
3	083	075	008	63	101	089	012
4	084	076	008	64	101	086	015
5	085	078	007	65	101	086	015
6	085	068	017	66	101	085	016
7	088	082	006	67	101	082	019
8	088	080	008	68	102	090	012
9	088	078	010	69	102	083	019
10	089	076	011	70	103	089	014
11	089	075	014	71	103	078	025
12	090	077	013	72	105	096	009
13	090	078	012	73	105	095	010
14	091	087	004	74	105	093	012
15	093	089	004	75	105	092	013
16	093	081	012	76	105	089	016
17	093	081	012	77	105	087	018
18	093	082	011	78	105	087	018
19	094	082	012	79	105	086	019
20	094	080	014	80	105	083	022
21	095	086	009	81	106	087	019
22	095	084	011	82	107	093	014
23	095	085	010	83	107	091	016
24	095	082	013	84	107	084	023
25	095	083	012	85	107	090	017
26	095	080	015	86	108	088	020
27	095	080	015	87	108	093	015
28	095	075	020	88	108	095	013
29	095	085	010	89	108	085	023
30	096	087	009	90	109	091	018
31	096	080	016	91	110	097	013
32	096	079	017	92	110	095	015
33	096	084	012	93	110	095	015
34	097	090	007	94	110	089	021
35	097	091	006	95	111	106	005
36	097	087	010	96	111	100	011
37	097	083	014	97	111	097	014
38	097	084	013	98	111	088	023
39	097	081	016	99	111	085	026
40	098	094	004	100	113	103	010
41	098	087	011	101	113	098	015
42	098	085	013	102	113	100	013
43	098	085	013	103	113	099	014
44	100	092	008	104	114	100	014
45	100	090	010	105	114	099	015
46	100	090	010	106	115	091	024
47	100	090	010	107	117	108	009
48	100	090	010	108	118	100	018
49	100	089	011	109	118	097	021
50	100	089	011	110	119	100	019
51	100	088	012	111	119	108	011
52	100	087	013	112	121	098	023
53	100	087	013	113	121	089	032
54	100	087	013	114	121	105	016
55	100	087	013	115	121	115	006
56	100	085	015	116	121	111	010
57	100	085	015	117	121	111	010
58	100	084	016	118	121	108	013
59	100	083	017	119	122	110	012
60	100	075	025	120	122	100	022

TABLE II—CONT'D

NUMBER	GM OF GLUCOSE FOR EACH 100 C.C. OF BLOOD			NUMBER	GM OF GLUCOSE FOR EACH 100 C.C. OF BLOOD		
	FOLIN WU	BENEDICT	DIFFERENCE		FOLIN WU	BENEDICT	DIFFERENCE
111	123	109	014	158	195	170	025
112	123	105	017	159	204	183	021
123	125	078	047	160	206	184	022
124	125	105	020	161	208	196	012
125	125	105	020	162	210	194	016
126	125	098	027	163	212	210	002
127	125	086	039	164	214	196	018
128	127	108	019	165	214	196	018
129	128	121	007	166	214	190	024
130	129	117	012	167	216	210	006
131	131	111	020	168	216	206	010
132	136	121	011	169	216	193	023
133	138	132	006	170	222	208	014
134	145	136	009	171	222	216	006
135	146	138	008	172	230	214	016
136	148	134	014	173	230	210	020
137	150	148	002	174	238	218	020
138	150	129	021	175	238	182	056
139	152	148	004	176	240	212	028
140	153	153	000	177	242	234	008
141	153	138	015	178	242	232	010
142	153	132	021	179	250	238	012
143	153	137	016	180	250	212	038
144	156	120	036	181	254	250	004
145	157	129	028	182	258	238	020
146	160	130	030	183	258	226	032
147	160	157	003	184	266	264	002
148	160	152	008	185	266	222	044
149	164	154	010	186	296	204	092
150	168	160	008	187	302	296	006
151	170	166	004	188	302	274	028
152	172	168	004	189	332	332	000
153	178	168	010	190	352	320	032
154	182	160	022	191	360	340	020
155	182	174	008	192	376	350	026
156	184	180	004	193	400	392	008
157	186	184	002	194	412	400	012

Thus it may be seen that Benedict's new procedure invariably yields lower results than the Folin Wu procedure. The differences however are not as great as those reported by Benedict the average difference for the total series being 14.5 mg per cent.

In the ninety cases in which the values determined by the Folin Wu method are below 0.110 gm for each 100 c.c. of blood and may be considered as normal or subnormal values, the average difference is 13 mg per cent.

There are eighteen samples with sugar values determined by the Folin Wu method of 0.100 gm for each 100 c.c. of blood. The Benedict method gave results averaging 12.4 mg per cent lower although in one case the difference was only 2 mg per cent and one sample differed 25 mg per cent.

CONCLUSIONS

1. The modified copper solution of Folin Wu as reported by Benedict yields results considerably lower than by the original Folin Wu procedure. The differences are not consistent in our hands, but on the average are approximately 12 mg per cent lower in a series of 194 consecutive samples.

2 The recovery of glucose added to blood filtrates is more satisfactory by the Benedict modification

3 Routine procedures will be continued on the second modification of Benedict

REFERENCES

- ¹Benedict, S R The Determination of Uric Acid in Blood, Jour Biol Chem, 1922, li, 187 207
- ²Benedict, S R The Determination of Blood Sugar, Jour Biol Chem, 1925, lxi, 207 213
- ³Folin, Otto The Determination of Sugar in Blood and in Normal Urine, Jour Biol Chem, 1926, lxxvii, 357 370
- ⁴Folin, Otto, and Berglund, Hilding A Colorimetric Method for the Determination of Sugars in Normal Human Urine, Jour Biol Chem, 1922, li, 209 211
- ⁵Folin, Otto, and Wu, Hsien A System of Blood Analysis Supplement I A Simplified and Improved Method for Determination of Sugar, Jour Biol Chem, 1920, xli, 367 374

VOLUME MEASUREMENT OF BLOOD PLATELETS*

By C M VAN ALLEN, M D, IOWA CITY, IOWA

BLOOD platelet estimation by volume is a departure from current practice, but the following method is presented because of its relative simplicity and reliability, and because of the significance of platelet volume in itself

PRINCIPLE

If a specimen of blood, freshly drawn and mixed with an anticoagulant, be allowed to stand, the corpuscles will sink gradually and leave a supernatant fluid of whitish, ground-glass appearance in which platelets remain uniformly suspended for hours As is well known, the platelet content of this fluid is the same per unit volume as was that of the whole specimen before sedimentation, and this fact has been made use of in the platelet counting method of Thomsen¹ and modifications by Giam,² Schenk and Spitz,³ and Reimann⁴ In the present procedure, instead of counting the platelets in the supernatant fluid from such a preparation, they are extracted from it by centrifugalization and then volume is measured

APPARATUS

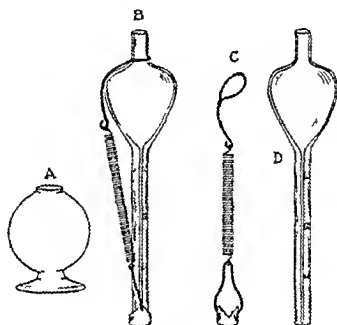
Instruments† required are the "thrombocytocrit," sedimentation chamber, 10 c c Record syringe, and a centrifuge capable of 3500 r p m The thrombocytocrit (Fig D) is a glass capillary tube with overlying chamber of 6 c c capacity The capillary bore is of 0.03 c c capacity, and the tube is graduated in a finely divided scale Its lower end may be closed by applying the accompanying spring sealing clip⁵ (Fig C) in the manner illustrated (Fig B) The sedimentation chamber (Fig A) is a spherical flask of 20 c c capacity

*From the Laboratories of the Rockefeller Institute for Medical Research New York City and the University Surgical Clinic Munich Germany
Received for publication July 30 1926

†The instruments may be obtained from Arthur H Thomas Co Philadelphia and Firma Arno Haak Jena Germany

METHOD

Six c.c. of a 13 per cent solution (isotonic⁶) of sodium oxalate is drawn into the syringe, the air bubble being excluded, and 4 c.c. of blood is added by venipuncture. This is deposited in the sedimentation chamber and more solution added, to 20 c.c. The chamber contents are then mixed thoroughly with the syringe and are set aside for three and one half hours for sedimentation. By means of syringe and needle 5 c.c. of the supernatant fluid is carefully removed from the chamber, including a little of the corpuscular sediment, enough to give to the fluid a faintly reddish tint. This is then deposited in the thromboeytoerit, the sealing clip having been already applied and the instrument is centrifugalized at about 3500 r.p.m. for one and one half hours (see below). On examination, the capillary of the thromboeytoerit will be found partly filled with sediment arranged in two sharply defined strata below, a column of red substance representing the corpuscles included in the



Figs. A-D

fluid, and, above this, an ivory white column composed of platelets. The extent of the white column is then read from the scale indicated in parts of a cubic centimeter. By shifting the decimal point in the figure obtained two places to the right, the result will indicate the percentage of platelets by volume in whole blood.

RESULTS

Data obtained from normal individuals of three species are given in the table. Experimental work in which the method has been used is being reported elsewhere.

TECHNICAL PRECAUTIONS

The blood must be taken directly and rapidly from the circulation and its immediate and thorough mixture with the anticoagulant assured, in order to prevent the platelet lysis which starts in blood directly after shedding. Smaller amounts of blood than that specified may be employed if desired and the readings correspondingly adjusted. Sedimentation should not be contin-

ued longer than four hours. A small amount of corpuscles is included with the fluid for centrifugalization, in order that the bottom of the capillary may be filled with this sediment (red) and the stratum of platelets elevated to a position where its limits can easily be read. After adding the fluid, the thrombocytoerit should be centrifugalized as soon as possible. Counterbalancing must be accurately attended to as regards both the amount and the distribution of weight because of the unusual shape of the instrument. As counterweight a second thrombocytoerit filled with water serves most efficiently. The duration of centrifugalization necessary to procure complete packing of the platelets in the capillary bore depends upon the particular centrifuge used (radius of rotation), and is to be determined, to begin with, by reading the

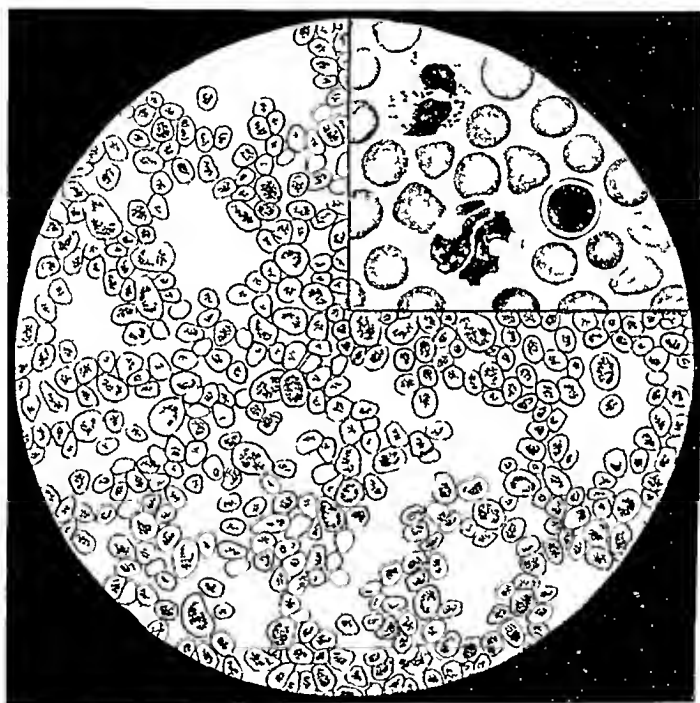


Fig E—Drawing of platelet substance (high oil magnification) as removed from thrombocytoerit and stained. Insert for comparison blood corpuscles at same magnification.

same specimen repeatedly, i.e., after one hour's centrifugalization and at fifteen-minute intervals thereafter. A typical result is 0.80 per cent, 0.75 per cent, 0.73 per cent, 0.72 per cent, 0.72 per cent, 0.71 per cent, 0.71 per cent, 0.71 per cent, here a period of one and three-fourths hours has reduced the reading to 0.72 per cent, which is close enough to its final value, and this amount of centrifugalization may be adopted for routine use. Particular care must be given in cleaning the capillary of the thrombocytoerit, for platelets tend to adhere to the wall of an unclean tube and form a thin coating on the glass above the white column, which, if extensive, vitiates the reading. Before each use the instrument should be immersed in cleaning fluid (sulphuric acid-sodium dichromate solution) for six hours or more, then rinsed carefully and dried. The oxalate solution should be clear.

TABLE I

SPECIES	NUMBER EXAMINED	NORMAL PLATELET VOLUME (PER CENT OF WHOLE BLOOD)	
		RANGE	AVERAGE
Man	15	0.35-0.67	0.49
Dog	15	0.70-1.50	1.04
Rabbit	15	0.40-0.72	0.53

COMMENT

The nature of the white substance which is measured in the thrombo-cytoerit, when removed from the capillary and stained is shown in the drawing (Fig E). For comparison the insert shows blood corpuscles of the same magnification. White blood cells are to be found among the platelets but are so rare as to have no volumetric significance, even under the circumstances of leucocytosis. The homogeneous consistency of the platelet substance is also demonstrated by the fact that it fails totally to appear in the capillary, when test is made of the blood of an animal treated effectually with a thrombo-cytolytic agent. The considerable individual variation here reported in normal blood platelet volume is in agreement with that known in normal counts of these structures. Platelet volume measurements run parallel, for the most part, with values obtained by counting, but the parallelism is not complete, nor is that to be expected since the size of the platelet may vary considerably in dispersal and between species. Measurement of the volume relations of the platelets in the blood would seem to be of distinct advantage in estimating thrombo-cytic function, since apparently platelets act quantitatively in the blood coagulative process by virtue of their chemical composition rather than as individual elements and assist also in the control of bleeding (thrombosis) by mass accumulation.⁶ The method is entirely mechanical microscopy and the personal element therein being eliminated. It permits the saving of considerable time where a number of tests are required and sedimentation of several specimens is done simultaneously. The thrombo-cytoerit may be used, also, to measure the amount of any finely divided material in fluid suspension, as the bacterial content of vaccines or for the purpose of obtaining blood platelets in pure deposit for study.

REFERENCES

- ¹Thomsen, Oluf. Acta med Scand, 1920 lxxv, 501.
- ²Gram H. C. Acta med Scand, 1920 xli, 1.
- ³Schenk, P. and Spitz S. Med Klin 1921 xlv, 381.
- ⁴Reimann, H. A. Bull Buffalo Gen Hosp, 1924 ii, 17.
- ⁵Van Allen, C. M. Jour Am Med Assn Dec 26 1920 lxxxi, 2077.
- ⁶Van Allen C. M. Jour Lab and Clin Med September 1921 x, 1027.
- ⁷Reimann H. A. Jour Exper Med 1924 xl, 553.
- ⁸Duke, W. W. Arch Int Med 1912 x, 445.

A NOTE ON THE KOCH AND McMEEKIN METHOD FOR
THE DETERMINATION OF NITROGEN, WITH SPECIAL
REFERENCE TO THE NONPROTEIN NITROGEN
OF BLOOD AND URINE*

BY H A DAVENPORT, M D, ST LOUIS, MISSOURI

THE direct nesslerization, or micro-Kjeldahl method for the determination of nitrogen described by Koch and McMeekin employs 30 per cent hydrogen peroxide (Meick's superoxol or Kahlbaum's perhydrol) to assist in the oxidation of carbonaceous material formed in the process of digestion with sulphuric acid. Since 3 per cent hydrogen peroxide (U S P) is more readily available than the 30 per cent peroxide, and may be handled with impunity, it seemed desirable to determine whether it might be satisfactorily substituted in the determination of nonprotein nitrogen of blood and urine.

Most 3 per cent peroxides contain one-fifth grain of acetanilid per fluid ounce, so experiments were made to determine the extent of error caused by this substance. One-tenth of a gram of acetanilid was added to 1 c c of half-concentrated sulphuric acid and digested as in nonprotein nitrogen determinations. When diluted and nesslerized, no color was produced. If, however, acetanilid free peroxide was added to such a digesting mixture, nitrogen was liberated as ammonia. Duplicate 5 c c portions of four different brands of U S P peroxide were then digested, diluted, and nesslerized, and read against a standard of equal volume which contained 0.25 mg. of nitrogen. A value of 0.046 mg. per c c was obtained for three of the samples, while the fourth (Parke, Davis and Co.) gave too little color to be read. No acetanilid content was declared on the label of the latter. Since the theoretical amount of nitrogen available from peroxide with the usual acetanilid content is approximately 0.042 mg. per c c, it appears to be liberated quantitatively. Therefore, a correction of 0.002 mg. N per drop (20 to 25 drops to the c c) of peroxide used will bring the accuracy of results within the limit of error of the colorimetric method.

A series of experiments on blood filtrates, on urine, and on a standard urea solution gave satisfactory results when 5, 10, and 15 drops of peroxide were used, provided the above correction were applied. Three drops were often sufficient to clear blood filtrate.

The peroxide should be dropped directly into the hot acid mixture, and not allowed to run down the side of the tube. If it is added in this manner immediately or within five to ten seconds after removal of the flame, a minimum amount is required.

*From the Department of Biochemistry, Washington University Medical School.
Received for publication July 19, 1926.

Example of the application of the correction for U S P peroxide (containing acetanilid)

1	5 cc blood filtrate digested with 1 cc half concentrated H SO ₄ and addition of 5 drops nitrogen free 3 per cent peroxide	N found	0 160 mg
	equivalent to N per 100 cc of blood		32 000 mg
2	Same as above except that 5 drops of U S P peroxide were used—	N found	0 176 mg
	Less correction of 0 002 mg per drop 0 010 = 0 166 mg		
	or N per 100 cc blood		33 000

SUMMARY

Ordinary 3 per cent hydrogen peroxide (U S P) instead of the 30 per cent solution may be used to clear the sulphuric acid digests of blood and urine in micro nonprotein nitrogen determinations by the Koch McMeekin procedure, provided a correction be made for nitrogen derived from the acetanilid. With this slight change, this method is in our hands preferable in that the nesslerized solutions are always clear and the separation of silver, which often occurs with the phosphoric acid mixture is avoided.

REFERENCE

Koch, F C., and McMeekin, T L. A New Direct Nesslerization Micro Kjeldahl Method and a Modification of the Nessler Folin Reagent for Ammonia Jour Am Chem Soc., 1924, xlii 2086

A SIMPLER METHOD FOR THE PREPARATION OF POTASSIUM PYROGALLATE SOLUTION FOR METABOLIC RATE DETERMINATIONS*

By FRANCIS F SCHWENTKER B S SCHENECTADY, N Y

MOST basal metabolic rate determinations include in their technique the analysis of expired gases. For this purpose Haldane gas analysis tubes are generally used, in which the oxygen content of the gas is adsorbed by a potassium pyrogallate solution. Definite directions for the preparation of this solution are given by Haldane¹ and the finished product adsorbs the oxygen faultlessly, but its preparation is difficult and uncertain. At times it seems impossible to obtain the intermediate potassium hydroxide solution with a density of 1.55 at room temperature and often, the finished product having been obtained, it persists in crystallizing instead of remaining a deep wine color.

Following are directions for the preparation of this potassium pyrogallate solution which eliminate much of the difficulty usually experienced and have the decided advantage of always producing the required solution. In addition, the finished product is identical with that called for by Haldane

From the Laboratory of the Ellis Hospital Schenectady N Y
Received for publication August 20 19 6

To 600 gm of stick potassium hydroxide (not purified by alcohol),* 300 cc of distilled water are added and the hydroxide dissolved by placing the mixture on a cold water-bath and heating the bath to boiling. As soon as complete solution has been effected, the volume is roughly measured.

The density of this hot solution is then determined by quickly weighing 100 cc in a volumetric flask and should be 1.517 at the temperature the solution will assume if weighed sometime within three minutes after removal from the water-bath. If it has not this density, the solution must be poured back with the remainder and after replacing on the water-bath, potassium hydroxide or water added according to whether the density is less or greater than 1.517.

When the correct density has been attained, the hot solution is poured into a glass stoppered bottle (with greased stopper) containing 9.7 gm of Merck's pyrogalllic acid for each 100 cc of the potassium hydroxide. The resulting mixture will be the required brown-green potassium pyrogallate solution which, after cooling, gradually assumes a deep wine color. It should be at least a month old before using, although, as Boothby and Sandiford² point out, the aging can be hastened by exposing to the air for a few minutes.

It will be found that if the above method is followed the density of the potassium hydroxide solution at the first determination is usually somewhat higher than 1.517. The amount of water necessary to dilute to the proper density can be quickly and easily approximated by the following formula:

$$\text{No cc diluting water necessary} = \frac{100 (\text{Density}) - 151.7}{51.7} \\ \text{per 100 cc solution}$$

The density of the solution will usually be found correct after the addition of the volume of water computed by the above formula, but the actual density should be more accurately determined by weighing.

REFERENCES

- ¹Haldane, J. S. *Methods of An. Analysis*, London, 1912, Griffen.
- ²Boothby, W. M., and Sandiford, I. *Laboratory Manual of the Technique of Basal Metabolic Rate Determinations*, 1920, W. B. Saunders Co.

*The potassium hydroxide purified sticks of Merck's are according to the manufacturer not purified by alcohol and are suitable for use in this preparation.

NEW APPARATUS FOR MEASURING THE SPONTANEOUS MOTILITY OF ANIMALS*

By CURT P. RICHTER, PH D., AND GING H. WANG, PH D., BALTIMORE MD

DESCRIPTIONS have been given in previous papers of cages used in investigating the different factors involved in the production and modification of spontaneous activity of animals (rats, etc.), (Richter¹ 1922, 1926), (Waug, 1923, 1925² *) Because these cages had a number of serious defects which curtailed their usefulness for this type of work, we have designed a new cage very much simpler in both construction and operation.

A photograph of three racks of these cages is shown in Fig 1. The racks, seventy two inches long seventy six and one quarter inches high, and fourteen and one half inches wide, are made of three quarter inch angle iron with a one eighth inch galvanized iron partition. Each one contains sixteen cages arranged in four tiers. The cage attached to the partition, consists of a living compartment with a food box and a watering tube and a revolving drum with a device for registering the revolutions. The living cage communicates with the revolving drum through a circular hole three inches in diameter in the partition. The dimensions of the individual cages and of the surrounding framework are shown in Fig 2.

The living compartment is built entirely of one half inch mesh wire cloth, with the side toward the partition open. In order to divert practically all of the animal's activity to the drum where it can be measured this living cage is made as small as possible—eleven inches long, five inches high, and three inches wide. It can be easily removed from the two hooks fastened to the partition to support it. The small recess built in one end to hold the food cup is covered with a special arrangement to prevent the spilling of food, and is so constructed that the cup can be removed from the outside without disturbing the animal. An inverted watering tube, graduated in cubic centimeters (Richter 1926) is fastened to the cage with a phosphorus bronze clip at the end opposite to the food box.

The revolving drum is made of one eighth inch mesh wire cloth, six inches wide mounted on a wooden disc thirteen inches in diameter and one half inch thick. The disc is attached to the axle of a ball bearing bicycle hub and the hub is firmly bolted to the partition. A small hole in the partition accommodates the other end of the axle.

The revolutions of the drum are registered automatically by means of a device shown in Fig 2. A small brass arm one half inch in length is fastened to that end of the axle which protrudes through the hole in the partition. A joint connection is made between this arm and the lever of a cyclometer by means of an aluminum rod. Thus, when the drum revolves

From the Psycho Biological Laboratory, Johns Hopkins Hospital, Baltimore, Md.
Received for publication July 11, 1936

the aluminum rod fastened excentrically to the axle of the drum causes the lever of the cyclometer to be raised and lowered. In this way each revolution of the drum, clockwise or counter-clockwise, is registered. The cyclometer is attached to the partition in such a way that it can be read with little effort, and the whole arrangement is very simple in construction and operation. It is a modification of the method used by Stewart⁷ (1898).

The racks are made very firm so that one animal may not stimulate another through vibrations set up by the revolving drum. Thus far we have found no indication of such interstimulation.

In designing these cages and stands we made a special effort to insure

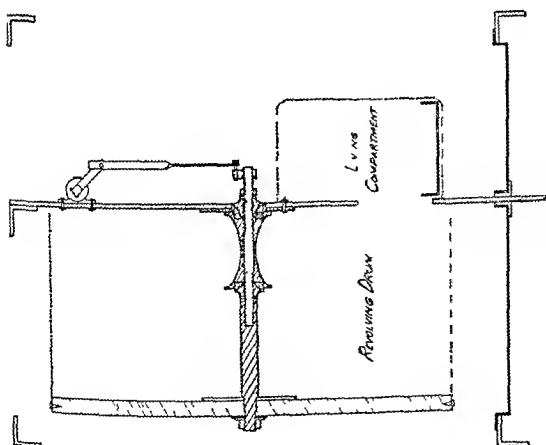
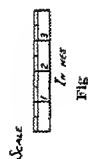
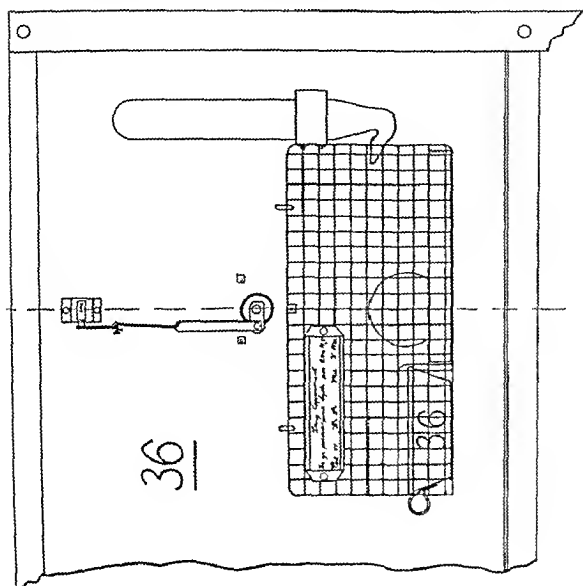


Fig 1

that they might be easily and thoroughly cleaned. Practically all parts of the cages can be detached, and the stands themselves are almost entirely free from cracks and crevices in which dirt and vermin can collect. Pans filled with sawdust are provided beneath the living cages and the drums to catch the urine and feces. By changing the sawdust in the pans frequently almost all of the unpleasant odor usually present in animal rooms can be eliminated.*

This arrangement of cages and method of recording activity have the following advantages: (1) The cages are very compact so that a large num-

*The cages were made after a design by E. A. Kalstner, Baltimore. The watering tubes were made by Levitt and Ferguson Co., Baltimore.



ber can be kept in a relatively small space (2) They can be cleaned easily and frequently with slight disturbance (3) The registering device is very simple and cannot be easily thrown out of adjustment (4) The elimination of activity outside of the drum makes certain that most all of the animal's activity is registered That this method is successful is shown by the fact that the animal often runs as much as ten to fifteen miles in twenty-four hours, and occasionally as much as twenty-seven miles

REFERENCES

- ¹Richter Comp Psych Monographs, 1922, 1, No 2, p 155
- ²Richter Jour Exper Zool, LV, 397-418
- ³Wang Comp Psych Monographs, 1923, 11, No 6, p 127
- ⁴Wang Am Jour Physiol, 1925, LXXI, 729-735
- ⁵Wang Am Jour Physiol, 1925, LXXI, 736-741
- ⁶Wang, Richter, and Guttmacher Am Jour Physiol, 1925, LXXII, 581-599
- ⁷Stewart Am Jour Physiol, 1898, 1, 40-56

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILGUFFE M.D. ABSTRACT EDITOR

Martin O. J. and Lepper E. H. A Micro method for the Estimation of the Hydrogen Ion Concentration of Capillary Blood. *Biochem Jour* 1926, 21, 37

Requirements—1 A freshly prepared saline solution containing 0.7 per cent NaCl and 0.2 per cent potassium oxalate or 0.2 per cent sodium fluoride. Shortly before use, one volume of 0.02 per cent phenol red is added to 10 volumes of saline and the P_H adjusted to about 7.5 with N/50 NaOH containing the same amount of indicator. It is kept in a hard glass bottle protected from CO₂.

2 A set of standard tubes made of resistance glass 7 cm long and 2.5 mm internal bore. These are filled with M/15 phosphate solutions of P_H varying from 7.3 to 7.6 at intervals of 0.05, to which one tenth volume of 0.02 per cent phenol red has been added, and sealed off. These standards must be made up frequently as they fade.

3 A number of pieces of resistance glass tubing about 8 cm long, and of 2.5 mm internal bore drawn out at each end to capillary dimensions so as to leave about 7.5 cm from shoulder to shoulder. The glass tubing must be selected and prepared as described below under "special precautions."

Procedure—A mark is made on one of the pieces of resistance tube at 0.5 cm from the shoulder of one end, A. The tube is then filled from end B up to this mark with the saline containing phenol red and placed ready in a horizontal position. A large drop of blood is obtained from the finger or ear by a deep puncture and the end B of the tube, already filled with saline, is placed in it. End A is slightly depressed and the blood is allowed to flow in, pushing the saline in front of it until the saline reaches the beginning of the capillary at end A. When this has occurred end B is wiped dry and end A sealed in a peep flame. As this end cools, blood is sucked into end B which is also sealed either in the flame or with sealing wax. The blood and saline are at once mixed by rotating the tube between the fingers to avoid clotting. The corpuscles are then separated by spinning in a hand centrifuge for a couple of minutes and the color of the mixture of saline and plasma compared with that of the phosphate standards.

The matching is done by holding the tubes in a good light at an angle of 45° to a piece of white paper lying on the bench. The tubes should be held by the capillary ends so that they are not warmed by contact with the hand. Greater accuracy is obtained if they are placed in test tubes of water at 18° containing a slip of white filter paper as background.

If the unknown is found to lie between say 7.35 and 7.4 another standard of P_H 7.375 may be made up and the relation of the unknown to this determined. With a little practice and P_H around 7.4, it is possible to place the unknown between two standards 0.025 P_H apart. The range of P_H over which such fine adjustment can be made will depend on the color vision of the individual worker. For most purposes, however, intervals of 0.05 in the P_H of the phosphate standards are close enough. This enables a determination to be made within $\pm 0.02 P_H$.

Special Precautions—1 The stock phosphate solutions must be made up with the greatest accuracy attainable. They must be kept in hard glass bottles and protected from evaporation and from CO₂ when withdrawing some by a trap of soda lime on the inlet tube.

2 The indicator should only be added to the saline solution shortly before use and the P_H adjusted to 7.5.

3 The whole reliability of the method depends on the small glass tubes not furnishing alkali. Only resistance glasses are suitable and these must be individually tested as described below, as glass from the same batch varies.

Pieces of tubing are washed and left standing in boiled distilled water containing phenol red. If after a few hours the contents of the tubing become pinker the glass is unserviceable. If no change in color occurs, the tubing is dried, cut into lengths and the ends drawn out.

As most resistance glass yields alkali temporarily after being melted in the flame, the tubes are rinsed in distilled water and left to soak for some hours in boiled out distilled water containing phenol red. If no pink color develops inside the tubes they are fit for use. They are rinsed with distilled water and dried with alcohol. *They must not be dried in a hot oven.*

4 In filling the tubes the minimum of air necessary for easy sealing should be left and entrance of CO_2 from the peep flame during sealing avoided.

5 Unless NaF is used, separation of the corpuscles and comparison with the standards should be undertaken forthwith. With oxalate, the P_H gradually falls from glycolysis. At room temperature no change can be detected after an hour, but at 38° a fall is discernible after a quarter of an hour. The use of fluoride as anticoagulant inhibits glycolysis (Evans). If 0.2 per cent NaF is employed the P_H generally remains constant for twelve hours at 18° . This is a convenience in clinical work, as the sample of blood can be taken and mixed with the saline at the bedside and the remaining stages proceeded with at leisure.

6 The colorimetric observations must be made at or about 18° . The P_H attributed to a particular color of the indicator in Sorensen's various phosphate mixtures is only valid for 18° so that if the comparison is made at another temperature the result will be incorrect. When using phenol red the error from this cause is of no great moment provided the temperature change does not exceed $\pm 3^\circ$, as this would lead to a difference of only 0.015 in P_H . Greater accuracy is obtained if the standard tubes and the blood containing tubes are placed in test tubes of water at 18° as described above.

If Cullen's correction of -0.2 is applied to the P_H of human blood determined colorimetrically at 18.20° the result will closely approximate the P_H of arterial blood at body temperature.

Fowweather, F. S. Determination of the Amount and Composition of Fat of Feces. I. Investigation of a "Wet" Method and Comparison with the "Dry" Method. *Brit Jour Exper Path*, February, 1926, vii, 7.

A comparison of Saxon's method for the fresh specimen with the method described by the dry method of Cammidge.

The technique of the "wet" method is as follows.

The sample is first thoroughly mixed, using a pestle and mortar if necessary to ensure uniformity. If liquid, a portion of the stool weighing about 5 gm is poured into each of three stoppered weighing bottles, and the weight of each portion obtained. One weighing bottle is placed in a steam oven and dried to constant weight in order to determine the percentage of dry matter contained in the stool. Each of the remaining portions is washed into a 100 c.c. graduated glass stoppered cylinder of uniform width throughout. To the contents of one cylinder 3 c.c. of concentrated hydrochloric acid are added, and then distilled water to 30 c.c. The contents of the second cylinder are diluted to 30 c.c. also without the addition of any acid. If the stool is solid a quantity of about 10 gm is placed in a weighed porcelain evaporating dish or crucible provided with a glass rod 2 to 3 inches in length, flattened at one end. Having obtained the combined weight of dish (and rod) and feces, a quantity of the latter weighing 2 to 3 gm is withdrawn on the flattened end of the rod and introduced into the cylinder by touching the cylinder wall with it below the ground portion at the mouth. The dish and contents together with the rod, with any feces still adhering to it, are then reweighed. A second weighed quantity of feces is similarly transferred to the second cylinder, after which the dish, rod and contents are dried to constant weight in the steam oven. Acid and distilled water are added to the cylinders as in the case of liquid stools. To each cylinder are now added 20 c.c. of ether, and the cylinders vigorously shaken for five minutes. Then, after standing for a few moments, 20 c.c. of 95 per cent alcohol are added to the neutral cylinder and 17 c.c. to the acid cylinder. The alcohol is mixed with

the other contents by giving the cylinders a sharp circular movement. Some rise of temperature occurs, and the cylinders are therefore stood in cold water in the sink for a short time, after which the contents are vigorously shaken for five minutes. The cylinders are then allowed to stand to allow the ether layer to separate. If separation does not occur readily gentle movement of the cylinder in a circular direction often helps, with the previous addition of a few drops of alcohol in most cases or ether in others. Experience will indicate the best means to adopt in any particular case, but once this has been obtained satisfactory separation need cause no difficulty.

The upper layer is blown off into a fat extraction flask by converting the cylinder into a wash bottle by the addition of a cork carrying glass tubes. The exit tube is turned up at the lower end to avoid any disturbance of the separating surface by upward currents in the liquid. The removal of the ether layer is made complete by addition to and removal from the cylinder of three successive 5 cc quantities of ether.

A second extraction is then made by adding another 20 cc of ether to each cylinder, shaking for five minutes, separating and transferring the ether layer as before. From the combined extracts in the flask the solvent is evaporated, and the residue is dried and dissolved in petroleum ether. The solution is filtered into a weighed flask and the petroleum ether evaporated off. After drying again the weight of extracted fat is obtained. Titration of the fat of the neutral extraction in benzene solution by N/10 sodium alcoholate completes the analysis. All results are calculated as a percentage of the dry matter of the feces. They are thus strictly comparable with the results obtained by the dry method.

The wet method is regarded as more accurate and less likely to lead to erroneous clinical deductions.

Wile U J and Belote G H Syphilitic Alopecia Its Relation to Neurosyphilis Arch Dermat and Syph, April, 1920 xiii, 195

Syphilitic alopecia of the essential type has a high associated incidence of meningeal syphilis, as indicated by spinal fluid findings.

The absence of the accepted criteria in the spinal fluid cannot moreover, be accepted as absolute evidence of the absence of such involvement.

Microscopic study shows that the essential syphilitic alopecia is not due to any local pathologic disturbance of the scalp, or more specifically of the follicular apparatus. It is therefore not a true syphilid.

Clinical analogy affords the suggestion that it is due to endocrine dysfunction as a result of association and involvement of the autonomic nervous system.

Symptomatic alopecia representing a smaller group of the entire syndrome is a true syphilid, apparently caused by a perifollicular plasmaoma.

Jones H N and Wise L E Cellobiose as an Aid in the Differentiation of Members of the Colon Aerogenes Group of Bacteria Jour Bact, May 1926 xi No 5, 359

Cellobiose is a carbohydrate prepared from cellobiose octa acetate by acetolysis, the method of preparation in detail to be published.

When added to broth in 0.5 per cent concentration it serves as a ready and accurate means of differentiation between *E. coli* and *A. aerogenes* the latter forming gas and acid the former producing neither.

Thompson L. The Blood Agar Plate for Spore Forming Anaerobes Jour Bact, May 1926, xi, No 5, 305

Attention is called to the availability of the blood agar plate for the isolation and grouping of spore bearing anaerobes some being hemolytic, some producing methemoglobin, and others being without effect on red blood cells.

The media used was Huntton's hormone agar with 1.5 per cent agar, tubed in amounts of 10 to 12 cc. Plates were poured after serial inoculation and incubated in a Novy jar exhausted by a combination of hydrogen and alkaline pyrogallol methods. When hydrogen

alone was used the procedure was called the "single method", both methods combined were called the "double method"

The paper is illustrated with twenty three microphotographs and presents a tabular, tentative classification of anaerobes on the basis of their characteristics when grown on blood agar plates under anaerobic conditions

Greenbaum, S S Error of Basing Serum Diagnosis of Syphilis on the Kahn Reaction Alone Jour Am Med Assn, April 24, 1926, LXXXVI, 1273

Using both the Kolmer quantitative complement fixation test and the Kahn flocculation test as a routine, Greenbaum emphasizes that from three to four per cent of discrepancies occur. Either test may give occasional false negative reactions.

Occasionally, in treated cases, the Kahn test remains positive when the Kolmer test has become negative. The significance of these reactions has yet to be determined, although in a number of such instances the spinal fluid gave a positive Kolmer reaction.

Greenbaum believes that a dangerous error may be introduced if the Kahn test is relied upon as the sole means of serodiagnosis and that both tests should be used routinely.

Murphy, J B Observations on the Etiology of Tumors Jour Am Med Assn, April 24, 1926, LXXXVI, 1270

Using the same chicken tumor as was used by Gyc, his experiments were repeated using in detail the methods described by him but also using control cultures of normal tissues.

Anaerobic "cultures" of chick embryo and rat placenta proved just as effective as so called cultures of malignant tumors in activating chloroform treated filtrates of chicken sarcoma.

The necessity of assuming a cultivated living organism in the interpretation of Gyo's results is eliminated.

Mills, H R Comparison of the Kolmer Quantitative Test with the Routine Wassermann. Jour Florida Med Assn, February, 1926

A series of 1328 tests is reported the results of which corroborate the delicacy and specificity of the reactions with this technic previously reported by numerous observers.

Sauthgate, H W, and Carter, G Excretion of Alcohol in the Urine as a Guide to Alcoholic Intoxication. Brit Med Jour, March 13, 1926, 463

Within the range of forensic medicine there is no subject upon which medical evidence is more unsatisfactory than that pertaining to drunkenness.

Of late years this diagnosis has become of great moment in connection with motor accidents and, in the absence of incontrovertible evidence, is a matter of great difficulty.

The authors present experimental evidence indicating that

1 The alcohol in the blood is related to the amount of alcohol consumed when it is imbibed under constant conditions

2 The relation of alcohol in the blood to alcohol in the urine is a fairly constant one in many circumstances

3 The concentration of alcohol in the blood is related to the symptoms of intoxication of the central nervous system

From the experimental evidence demonstrating these propositions a practical method was devised for the calculation of the blood alcohol concentration at the time of arrest by deducing the blood concentration from the urine alcohol concentration.

As the collection of specimens of urine from a prisoner suspected of drunkenness may involve some difficulty a special cell is used the discharge pipe of the urinal of which passes to another cell and empties into a receptacle. Nothing is said to the prisoner and the bladder is emptied voluntarily and without suggestion.

The time of arrest is noted and also the time of the collection of the specimen which is examined at once by the method described below.

If the figure 130 be taken as the ratio of urine alcohol to blood alcohol the latter can be calculated. Knowing this figure and the time interval between the arrest and the passage of the specimen, the blood alcohol at the time of arrest can be calculated from the fact that the alcohol concentration in the blood falls at the rate of about 12 mg per hour per 100 grams of blood.

It remains to be determined by experiment, however, what blood alcohol concentration figure can be taken as the upper limit as regards the fitness of an individual to drive an automobile.

By the method described however the fact that an individual is "under the influence of liquor" may be rather definitely determined.

"The method of estimating alcohol in the urine is that of Cannon and Sulzer" (Cannon, R. K., and Sulzer, R. *Heart* London, April 5, 1924, 21: 148).

Reference to this paper gives a method for the estimation of alcohol in blood said to be applicable to other biologic fluids and which presumably is the method used by Southgate and Carter. The latter authors state that normal constituents of the urine do not interfere with the reaction and the presence of acetone bodies introduces only a minimal error 8 mg of acetone being equivalent to 1 mg of alcohol under experimental conditions. A routine test with Fehling's solution acts as a guard on this.

The method described by Cannon and Sulzer follows:

A known volume of blood is delivered directly on to two or three times its weight of anhydrous sodium sulphate distributed over the bottom of the distilling vessels, a special test tube type of which is described and illustrated. This is placed in a water bath at 40 to 50 C and evacuated through a tube containing a known volume of standard potassium dichromate and an equal volume of strong sulphuric acid.

Distillation is allowed to proceed for fifteen to twenty five minutes with the pump running, the vacuum then broken by opening the capillary inlet of the distilling flask and the absorption tube disconnected.

The contents are washed into a flask with sufficient water to dilute the sulphuric acid to less than 5 per cent, excess of 10 per cent potassium iodide solution added and the liberated iodine titrated with starch and sodium thiosulphate.

This titration subtracted from the thiosulphate titer of the volume of potassium bichromate used gives the amount of the latter required to oxidize the alcohol and from the factor (1 cc N/1 potassium bichromate = 1.15 mg alcohol) the amount of alcohol may be directly obtained.

Note—The amount of anhydrous sodium sulphate should be such as to give a semi liquid mass with the blood.

Piersol Geo. M. Bockus H. L. and Shay H. H. The Value of a Starch Iodine Reaction as a Test of Pancreatic Function. *Arch Int Med* March 1926 43: 431.

The following modification of the Bassler method was employed:

A duodenal tube was passed into the duodenum on an empty stomach preferably in the morning. When the tip of the tube was ascertained to be in the duodenum 100 cc of 5 per cent Witte peptone solution was injected through the tube as an activator to the pancreas. The tube was clamped off for five minutes then the duodenal contents were aspirated. The first fraction aspirated was used for the test because Bassler states that this contains the largest portion of pancreatic juice and represents the sudden liberation of the stored up secretion of the pancreas.

The following reagents were employed:

Solution A, prepared as follows. In a beaker 2 gm of cornstarch (Duryea) were placed to this were added 100 cc of cold distilled water. This was mixed thoroughly and then heated. Under constant stirring the mixture was brought to boiling and then cooled.

Solution B a 1 per cent sodium chlorid solution. The standard buffer solution mentioned in the original description of the test was omitted as it is now regarded as nonessential.

The standard Bassler reagent was then prepared by combining solutions A and B as

is the Wassermann reaction. This tends to be reduced to normal in the very early or acute cases, but seems to be uninfluenced and more likely to be fixed in the late cases than any other of the changed constituents of the spinal fluid.

Fisk, C. H., and Subbarow, Y. The Colorimetric Determination of Phosphorus. Jour Biol Chem, December, 1925, lvi, No. 2, 375.

Solutions needed

N/10 Sulphuric Acid—450 cc of concentrated sulphuric acid added to 1300 cc of water.

Molybdate I—2.5 per cent ammonium molybdate in N/5 sulphuric acid. Dissolve 25 gm of the salt in 200 cc of water. Rinse into a liter volumetric flask containing 500 cc of N/10 sulphuric acid. Dilute to the mark with water and mix.

Molybdate II—2.5 per cent ammonium molybdate in N/3 sulphuric acid. Prepared as above, but with only 300 cc of N/10 sulphuric acid. (To be used only with blood filtrates in the determination of inorganic phosphate.)

Molybdate III—2.5 per cent ammonium molybdate in water. As soon as any considerable amount of sediment (ammonium trimolybdate) has appeared in this solution, it should be discarded.

Ten per cent trichloroacetic acid—The quality of this reagent is of great importance. One brand tried contains some unknown impurity which retards the color development to a most pronounced degree. Merck's U. S. P. product is free from any such contamination, but contains a trace of phosphate. The amount of this must be determined in each sample or else the reagent purified by distillation.

Determination of impurity—Arrange three tall beakers of 150 cc capacity on a piece of white paper. Into one of these (A) put 100 cc of water. In a second beaker (B) mix 85 cc of water, 10 cc of Molybdate I, and 4 cc of 0.25 per cent aminoaphtholsulphonic acid; the result should be a solution practically as colorless as water, without a trace of blue (otherwise one or more of the reagents already added contains phosphate). To the third beaker (C) add 40 cc of the trichloroacetic acid solution, 45 cc of water, 10 cc of Molybdate II, and 4 cc of the sulphonic acid reagent, stirring thoroughly with a clean glass rod. Into B now run 1 cc of a dilute phosphate solution containing 0.005 mg of phosphorus per cc and mix well. Proceed in the same way, adding 1 cc of this phosphate solution at intervals of not less than two minutes, until B and C appear to have the same color when examined from above. The volume of phosphate solution which must be added to bring this about, multiplied by 0.05, is the correction (in mg per 100 cc) to be subtracted from the result in the analysis of blood.

Standard phosphate (5 cc = 0.4 MgP)—Dissolve 0.3509 gm of pure monopotassium phosphate in water. Transfer quantitatively to a liter volumetric flask, add 10 cc of N/10 sulphuric acid, dilute to the mark, and mix. The standard keeps indefinitely.

Fifteen per cent sodium bisulphite—The solution must be free from turbidity before it can be used. Freshly prepared sodium bisulphite solutions may not filter clear, in which case two or three days standing (before filtering) will be necessary. Keep well stoppered.

Twenty per cent sodium sulphite—Use the crystalline sulphite ($\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$). Dissolve 200 gm of this in 380 cc of water. Remove any suspended matter by filtration, and keep stoppered.

Aminoaphtholsulphonic acid, 0.25 per cent—Dissolve 0.5 gm of the dry powder (see next section) in 195 cc of 15 per cent sodium bisulphite, add 5 cc of 20 per cent sodium sulphite, stopper, and shake until dissolved. If the bisulphite solution is old, more than 5 cc of sulphite will be needed—in that event add more sulphite 1 cc at a time, shaking after each addition, until solution is complete. This reagent can be prepared in a few minutes (the powder need not be very accurately weighed), and if not left exposed to the air it should keep about two weeks. The solution is more stable the higher its acidity, hence no more sulphite should be added than is needed to dissolve the reducing agent.

1,2,4 aminonaphtholsulphonic acid—This may be prepared from β naphthol according to Fohn's directions, with a single alteration. The final product, after washing with cold water still contains some colored material. To remove this, the crystals on the filter, while still wet, should be further washed with alcohol as long as any color is extracted.

The reagent may also be obtained in satisfactory condition by one recrystallization of "technical" aminonaphtholsulphonic acid (Eastman Kodak Co.) as follows. Heat 1,000 cc of water to about 90° and dissolve in it 150 gm of sodium bisulphite and 10 gm of crystalline sodium sulphite. To this mixture add 15 gm of the crude sulphonic acid and shake until all but the amorphous impurity has dissolved. Filter the hot solution through a large paper (about 32 cm) cool the filtrate thoroughly under the tap and add to it 10 cc of concentrated hydrochloric acid. Filter with suction, wash with about 300 cc of water, and finally with alcohol until the washings are colorless.

The purified sulphonic acid should be dried in air with the least possible exposure to light, then powdered and transferred to a brown bottle.

Determination of inorganic phosphate in urine—Measure into a 100 cc volumetric flask enough urine to contain between 0.2 and 0.8 mg of inorganic phosphorus (usually 1 or 2 cc). Add water to bring the total volume to 70 cc followed by 10 cc of 2.5 per cent ammonium molybdate made up in N/3 sulphuric acid (Molybdate I) and 4 cc of 0.2 per cent aminonaphtholsulphonic acid. After the addition of each reagent the solution should be mixed by gentle shaking.

At the same time transfer to a similar flask cc of the standard phosphate solution (containing 0.4 mg of phosphorus) 65 cc of water and the same reagents that were added to the urine sample. Dilute the contents of each flask to the mark mix and compare in the colorimeter after five minutes. With the standard set at 20 mm, 8 divided by the reading will give the inorganic phosphorus content of the sample in mg.

Determination of inorganic phosphate in blood—Transfer to an Erlanmeyer flask four volumes of 10 per cent trichloroacetic acid. While the flask is being gently rotated run in 1 volume of blood, plasma, or serum—as the case may be—from a pipette calibrated for delivery (not contents). Close the mouth of the flask with a clean dry rubber stopper and shake vigorously a few times. Filter through an ashless paper.

Measure 5 cc of the filtrate into a tube graduated at 10 cc or a 10 cc volumetric flask. Add 1 cc of 2.5 per cent ammonium molybdate in N/3 sulphuric acid (Molybdate II) and finally (after mixing) 0.4 cc of the usual sulphuric acid reagent. Dilute to the mark and mix. The standard to be prepared as nearly as possible at the same time is identical with the standard used for urine (0.4 mg of phosphorus in a volume of 100 cc, or 0.2 mg in a 50 cc flask with half as much of each reagent), so blood and urine may be read against the same solution. It should be noted that the molybdate reagent added to the standard is always the one containing N/3 sulphuric acid (Molybdate I) and is different from that used for the blood filtrate. The purpose of this is to compensate for the high concentration of trichloroacetic acid in the filtrate.

The reading as with urine, may be made in about five minutes but it should be repeated a few minutes later if the color is particularly strong. To calculate the result in mg of phosphorus per 100 cc of blood or other fluid (the standard being set at 20 mm) divide 80 by the reading. From the figure so obtained subtract the correction for any phosphate which the trichloroacetic acid may contain.

An inorganic phosphorus content of 2 mg per cent is about the lower limit for convenient reading against the standard recommended and a weaker color such as would be obtained by using half as strong a standard cannot be read so accurately. Hence, as perhaps the least objectionable arrangement when the phosphate content of the blood is very low the addition of a known amount of phosphate to the filtrate is suggested. This may be done, when a low result can be anticipated before introducing the reagents. Otherwise if the reagents are already mixed with the blood filtrate and the color is seen to be unusually weak phosphate may be added then before diluting to the mark—the less delay of course the better, but any time within five minutes will do provided that twice as long a period is allowed before the final reading. A suitable amount of phosphorus to add is 0.016

five times the volume of absolute or 95 per cent alcohol and thoroughly mixed. The supernatant fluid is discarded after ten minutes and the cells are allowed to dry on a glass plate in the incubator at 37° C, or at room temperature, for about ten minutes. They are then ground into a fine powder by the mortar and pestle.

In the test, one volume of dried sheep corpuscles is mixed with about four or five volumes of active or inactivated human serum, shaken well and allowed to stand for a few minutes at room temperature. This mixture is then centrifugalized for five minutes at moderate speed and the serum drawn off. This procedure absorbs the natural anti-sheepamboceptor together with the hemoagglutinin and has no effect on the complement.

Bernheim, A. R. The Significance of Variations of Bilirubinemia. Arch Path and Lab Med, May, 1926, 1, No 5, 748

A study of 485 unselected cases using a modified Menlengracht test.

Observations of the variations in bilirubinemia are chiefly directed to the study of liver function, but bilirubin, besides being a product of the liver, is a normal constituent of the blood, and as such plays a rôle of some importance in a number of conditions not primarily concerned with the liver.

The accuracy of the test for the determination of the icterus index and the facility with which it is performed make it available not only for investigations in hospitals, but also for the use of the outside practitioner.

As a diagnostic and prognostic aid in a number of diseases, the determination of the icterus index is a procedure which may be said to hold equal rank with other laboratory tests, such as those for blood sugar, urea nitrogen and creatinine, with an advantage over these tests in that it is simpler to perform.

It is seen that under controlled conditions in which the liver is stimulated, blood sugar and bilirubin values bear a definite relation to each other. Explanations of these findings are presented with due recognition of the conjectural element involved, and with the hope that further evidence may either substantiate them or show why they are incorrect.

Hypobilirubinemia (below 2.3) may be noted where there is a reduction of red cells. In diabetes the icterus index is high (average 10).

Doan, C. A. Recognition of a Biologic Differentiation in the White Blood Cells. Jour Am Med Assn, May 22, 1926, LVIII, 1593

A study of white blood cells by a supravital technique in an attempt to find an explanation for the reactions noted in rabbits transfused with matched blood.

Technic—First, it is essential that the slides and cover glasses for the supravital preparations be free of grease and neutral in reaction (nonacid). Glassware is placed in a saturated solution of potassium bichromate in concentrated sulphuric acid for three or four days, and then washed in running tap water for twelve hours. It is rinsed in three changes of distilled water, the slides stand overnight in the last change. It is stored in 80 per cent alcohol. It is dried with clean, new gauze and flamed. If a dye is used (it is not essential), vital neutral red is recommended. Grubler's prewar, vital neutral red is made up in saturated solution in neutral absolute alcohol. Twenty-five drops of the saturated solution in 5 c.c. of absolute alcohol gives a nontoxic concentration of the dye for use in examinations of normal blood. The slides are flooded with the dye and then drained, they are allowed to dry in the upright position, which insures a thin even film of the dye on the slide. A film of blood made on such a slide, when kept in the warm box at 37.5° C, will show viable cells for as long a period as a film made without the dye. Moreover, the dye makes the individual cells much more easily visible and the identification of cell types more certain. The prime requisites, it is to be remembered, however, are motility of the cells and freedom from any technical cellular depressant. The weight of the cover slip will cause the drop of blood to spread to the desired extent, provided the glassware is clean. It is sealed with petroleum of a higher melting point than the temperature at which the examination is to be made. Either a warm box or a warm stage, preferably the former, may be used. The uniformly constant temperature conditions are an essential.

The cover slips are prepared in a similar way. They are flooded with the citrated or ovalated plasma or blood serum that has been secured for grouping the red blood cells, and drained to one corner in the vertical position the drop of plasma that forms there being carefully removed with a fine capillary pipette. This is necessary in order that the unevenness attendant on the drying of a large drop of plasma at that point may be avoided. From the finger or ear of the person whose white blood cells are to be tested a drop of blood is taken directly on the cover slip prepared with the dried serum and dropped gently on the slide. If the blood does not promptly spread into a thin even film slight manipulation from the side, never a pressure from above may be used. Immediately on the same slide and from the same or a subsequent drop of blood the control film is made with a plain cover slip. Both are sealed with petrolatum and under proper temperature conditions the control slip is looked at first to make certain of the viability of the leucocytes. It is the comparison or contrast between the number of viable cells in the two preparations on which depends the determination of the compatibility of the plasma in question. Ovalate and citrate anticoagulants in themselves do not affect the white blood cells in this test. However as will be seen when a blood is leucotoxic citrated or ovalated plasma is doubly destructive to the susceptible white cells. At certain times there are physiologic showers of the 'nonmotile' leucocytes described by Sabin and her coworkers in 1925 which must be differentiated from the white cells specifically affected by the incompatible blood plasma. Both by the difference in physical characteristics and by their presence and number in the control film, it is readily possible to eliminate such preparations as pseudopositives. If the control film, at any time contains a high percentage of the nonmotile leucocytes it is advisable to make a second duplicate preparation as this particular phase of the polymorphonuclear leucocyte is transitory and does not reflect the plasma effect so readily as does the actively motile form.

It was noted that certain serums have a more disintegrative effect than others on the white cells.

There seems to be a definite incompatibility between the blood plasma of certain individuals and the white blood cells of others as shown by simple in vitro tests.

A definite biologic classification of individuals according to white cell compatibility is difficult because of the fact that at least twenty seven different combinations are possible many of them not infrequent. Approximately 40 per cent of the group of forty persons investigated may be considered universal donors (group A). 5 per cent seem to have cells susceptible to all or nearly all plasma (group Z) nine different combinations are represented in the remaining 55 per cent to which no arbitrary group designation has been assigned at this time.

The added precaution of ascertaining the relative compatibility of plasma and white cells by direct matching preliminary to transfusion particularly in cases in which extreme weakness makes imperative a successful operation is advisable.

Lash A. F. and Kaplan B. Puerperal Fever Streptococcus Hemolyticus Toxin and Antitoxin. Jour. Am. Med. Assn. April 17 1926 LXXXVI 1197

Experiments with hemolytic streptococci isolated from the blood in puerperal fever. The toxic substance in the Berkefeld V filtrate of such cultures is a toxin because

- 1 There is a latent period between injection and reaction
- 2 It is heat labile
- 3 It is neutralized by antitoxin and not by non immune serum
- 4 Precipitin tests show the development of immune bodies by the toxin
- 5 Repeated injections of the toxin give rise to the production of a specific antitoxin

Nevin, M. Bittman F. E. and Hazen E. L. Unsuccessful Attempts to Cure or Prevent Tuberculosis in Guinea Pigs with Dreyer's Defatted Antigen. Ann. Rev. Tub. February 1926, xiii 114

The defatted antigen described by Dreyer produced no beneficial results either prophylactic or therapeutic in guinea pigs. On the contrary animals treated with the vaccine died earlier than the untreated controls.

BOOK REVIEWS

(Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building, Richmond, Va.)

*Radium Its Therapeutic Uses in General Practice**

THE author voices the regret so often expressed by radiologists who are dependent in great part for their work upon material referred from surgeons, that were radiotherapy given an untrammelled opportunity without antecedent surgical intervention, the results would be even better. This applies particularly to malignancy. Surgeons have not as yet reached the stage where they are willing to turn the case over to the radiologist for entire charge. If the case is still considered operable, even faintly so, the only chance that the x-ray or radium therapist has is along the line of preoperative and postoperative care. The time will come when he will have better opportunity to demonstrate the real values of his method of treatment.

The author includes sections devoted to the properties of radium, a brief historical recapitulation, description of induced radioactivity, discussion of technic and dosage. The major portion of the book is devoted to therapeutic methods and results in specified conditions. Radium has its place in the treatment not only of malignancy but in certain blood diseases such as leucemia and in the treatment of naevi, moles, papillomata, adenomata, warts, certain diseases of the uterus, goiter, actinomycosis, fibroids, acromegaly and other conditions. He finds that radium has more effect on metastases than on local recurrences or secondarily infected lymph glands. Malignant disease appears to be more susceptible to radium therapy when the lesion is "backed up" by bone. On the other hand, when the disease is in the bone itself the results are not as good. In prophylaxis he usually gives radium exposure two or three days prior to operation. A second course is given from four to six weeks following operation. Following breast operations he recommends the routine implantation of radium tubes in the region of the supraclavicular gland for a period of twenty four hours.

Squamous epithelioma of the tongue, esophagus and larynx is not very satisfactorily treated with radium, but the pain is usually relieved in these conditions. Epithelioma of the lower lip responds very readily. The author's results in carcinoma of the rectum have not been so satisfactory as in some other conditions, but they probably average up with other methods. Carcinoma of the cervix uteri responds splendidly. If taken early enough, the results appear better than by any other method and one is enabled to do away with the 20 per cent immediate operative mortality. The results with rodent ulcer are distinctly superior to those obtained by surgery.

It is surprising to read that the author does not report particularly good results in fibroid tumor of the uterus. This presumably is due to difficulty in access, for deep x-ray therapy gives excellent results.

There is a close parallelism between the results obtained by radium treatment and those obtained by a skilled x-ray therapist. With the somewhat greater flexibility in x-ray technic and with the advent of deep therapy, the x-ray will usurp much of the work that has been done by radium.

*Radium Its Therapeutic Uses in General Practice By G H Varley M D (Oxon)
Late Clinical Assistant to the Radiological Department St George's Hospital Cloth Pp 103
Oxford Univ Press 1924

*International Clinics March 1926**

THIS volume follows the time honored custom of *International Clinics* of presenting articles by recognized authorities from this country and abroad on subjects of diversified interest.

Dr. Reizenstein of Syracuse contributes a very good didactic discussion of the diagnosis and treatment of cardiac arrhythmias. It is well illustrated with electrocardiographic tracings. G. Paul La Roque, of Richmond, presents his views on the treatment of appendicitis in no uncertain terms. We are inclined to think of the surgical treatment of appendicitis as so thoroughly established as to permit of no further discussion. However, Dr. La Roque raises several pertinent questions, some of which will be found perhaps slightly embarrassing to the surgical enthusiasts who insist that with the establishment of the diagnosis operation must of necessity follow immediately irrespective of the various local factors that should call for individualization in treatment. At the same time several of the points raised should be interesting to the physician who precedes the surgeon and who too often when called upon to treat a patient with abdominal pain will prescribe a cathartic, a soft diet or even an enema. The article by Dr. La Roque alone should make the volume worth while.

We learn from Professor Plot of Munich that glandular parotitis is being treated with relapsing fever as well as with malaria. The former has the advantage that the supposed infection does not get out of control and that the virus for inoculation may be carried as a stock preparation in laboratory animals. It has the disadvantage that the patient having once recovered from relapsing fever cannot be reinoculated. The results with this treatment appear to compare very favorably with those following malaria.

In this as in other volumes of the series a section is devoted to electrotherapy and physiotherapy.

In the first volume for each year *International Clinics* incorporates a section on the progress of medicine during the preceding year. Under this heading, Doctors C. H. Coupland and Coupland bring out that while no outstanding, or spectacular single advance was made during 1925, the practical and clinical application of laboratory discoveries of the second order have distinctly improved our methods of treatment of certain common diseases such as gall bladder disease, paralytic postoperative obstruction, postoperative hypocalcemia and hypotension. They also point out that notwithstanding the valuable diagnostic procedures which the laboratory has given to medicine the trend has been back to careful physical diagnosis and the treatment of the individual rather than the disease from which he is suffering. The reaction against too much dependence upon laboratory methods alone continues. There has perhaps not been any diminution in the use of special laboratory tests but a more intelligent interpretation of their advantages and limitations and a greater consideration of the cost of time and money as compared to their actual values.

The subject of recent progress in surgery is covered by Balfour and Reid.

Prescription Notes†

A SMALL volume scarcely larger than a prescription pad bound in black leather which may be easily carried in the coat pocket. Most of the leaves are blank pages for the insertion of favorite prescriptions, new prescriptions that may be picked up from time to time, and notations concerning treatment. As an undergraduate student the reviewer had a little pocket notebook containing special prescriptions to which he added particularly during his intern year. Even today he refers to it for some long forgotten combination.

It must have been with the same idea in mind that Dr. Tatum made up the small volume under consideration. In the first 23 pages we find helpful information such as instructions for the proper construction and use of vehicles, general rules regarding incompatibilities, rules of dosage for children, weights measures and time, Lavoisier and a list of the more important preparations described in the U. S. Pharmacopoeia with their designs.

International Clinics A Quarterly of Illustrated Clinical Lectures and Especially Prepared Original Articles By Leading Members of the Medical Profession Throughout the World. Cloth Pp. 309 J. B. Lippincott Co. March 1926 Philadelphia.
†Prescription Notes By Arthur Tatum, M.D., M.D. University of Chicago. Cloth Univ. of Chicago Press 19

*The Clinical Interpretation of the Wassermann Reaction**

THE author requires no introduction to our readers. The reviewer would characterize this book so far as the complement fixation test for syphilis is concerned as the clinician's *vade mecum*. Only enough of the actual technic of the reaction is described so that the reader will have a clear understanding of the procedures applied. In every sense the work lives up to its title.

The author points out all possible sources of error, both in the hands of the serologist and those of the clinician and suggests the appropriate measures for avoiding these errors. The significance of anticomplementary reactions and of the provocative reaction are discussed. The Wassermann reaction in pregnancy and in the newborn is elucidated.

A chapter is devoted to the complement fixation reaction in diseases other than syphilis. The quantitative Wassermann reaction such as that elaborated by Kolmer is of particular value in treatment, since it gives a much more clearly defined picture of the progress of recovery.

We would recommend particularly to all physicians the reading of the chapter on the clinical interpretation of the cholesterol plus reaction, and that on the provocative reaction. It is our opinion that the latter is applied inaccurately by the clinician in from 60 per cent to 80 per cent of cases.

After giving a general discussion of the complement fixation reaction, the author describes those methods which are acceptable and reliable, and he mentions the more recent precipitation reactions. Concerning the latter he emphasizes particularly their shortcomings.

The last chapter deals with the proper methods of collection of specimens for the complement fixation test.

The great value of the book will be as a desk reference manual to use when in the individual case a question arises as to the significance or interpretation of a complement fixation reaction.

The Private Practitioner as Pioneer in Preventive Medicine†

SIR GEORGE NEWMAN in his Hunterian oration for 1926 reviews the contributions to the prevention of disease made by those practitioners of medicine who lived in

England in the Hunterian period or more broadly in the eighteenth century. Still early in the renaissance of modern medicine, these men were not alone interested in the cause of disease and in its cure, but even then they were taking active steps toward its prevention. Their interest was in epidemic disease such as plague, influenza, typhus, small pox and in such conditions as puerperal sepsis and chronic alcoholism. During the eighteenth century the more thoughtful and investigative of the British physicians introduced the principles of medical notification, of isolation, of fumigation and disinfection. They advocated an improved and enlarged dietary with restriction of alcoholic consumption.

They began the reformation of midwifery and first systematically attacked the problem of infant mortality. They lent their support to the establishment of dispensaries, hospitals, and medical schools. They laid the early foundations of immunology.

To us today the most outstanding feature of the contributions of the eighteenth century to preventive medicine in England was the discovery by Jenner of vaccination. The description which Dr Newman gives of the results of alcohol consumption in the early part of the century is most interesting in view of present conditions in the United States. "Drunkenness abounded. By 1720 we had become a nation of tipplers. The nobility patronized brandy, the well to do middle classes drank a newly introduced spirit called rum, the working classes imbibed a characteristic spirit known as British gin. The consumption of gin was enormous. When certain restrictions which had been temporarily imposed were repealed in 1733, England touched a lower depth of inebriety than ever known before or seen since. The prevailing intemperance was the most momentous event of the eighteenth century."

*The Clinical Interpretation of the Wassermann Reaction. By Robert A. Kilduffe. A.B. A.M. M.D. Cloth. Price \$2.50. Illustrated. Pp. 203. Lea & Febiger, Philadelphia, 1926.

†The Private Practitioner as Pioneer in Preventive Medicine. Being the annual oration of the Hunterian Society, 1926. By Sir George Newman. K.C.B. M.D. D.C.L. Pp. xi. Pp. 47. Oxford Univ. Press.

The Journal of Laboratory and Clinical Medicine

Vol. XII

ST. LOUIS, MO. DECEMBER, 1926

No. 12

Editor in Chief WARREN T. VAUGHAN, M.D.

Richmond, Va.

ASSOCIATE EDITORS

DE WISSE, JACK O., M.D.	CINCINNATI
PAUL G. WOOLLEY, M.D.	LOS ANGELES
J. J. I. MACLEOD, M.L.	TORONTO
W. C. MACCAEY, M.D.	ROCHESTER, MINN.
GERALD B. WEBB, M.D.	COLORADO SPRINGS
VICTOR C. MYERS, PH.D.	IOWA CITY
RUSSELL L. HAZEN, M.D.	ROSELAND, ILL.
JOHN A. KOLMER, M.D.	PHILADELPHIA
ROBERT A. KILGUSSE, M.D.	ATLANTA, GA.
A. PARKER HITCHCOCK, M.D.	MASSACHUSETTS
DEAN LEWIS, M.D.	BALTIMORE
GEORGE P. HEEPMA, M.D.	NEW ORLEANS
HAROLD D. CAYLOR, M.D.	ROCHESTER, MINN.

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright, 1926, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second Class Matter

EDITORIALS

The Epidemiology of Pneumonia

IN SPIKE of the vast amount of consideration and study which have been expended upon it, pneumonia still maintains the rank conferred upon it by Osler as 'Captain of the Men of Death.'

Lacking effective means for its specific treatment and in common with the general trend of modern medicine it would seem that the direction of attack must be, for the present at least oblique rather than frontal and directed toward the evolution of prophylaxis—if this be attainable.

Studies concerned with the spread of this justly dreaded infection are therefore, of great interest and such an analysis has recently been published by Roscnau, Felton and Atwater¹ which it is the purpose of this editorial to summarize.

Their study comprises 28 cases carefully typed and the records of 450 persons examined in an effort to define the sources and modes of infection with the pneumococcus.

¹ Roscnau, M. J., Felton, I. D., and Atwater, I. M. An Epidemiologic Study of Pneumonia and Its Mode of Spread. *Am. Jour. Hygiene*, May 1926, vol. 3, p. 16.

As pointed out by the authors, while man is the source and reservoir of the pneumococcus, the fact that pneumonia is a group of specific diseases complicates the study of its epidemiology as does the rôle of predisposing causes and accessory factors influencing its occurrence and virulence, all of which must be investigated before epidemiologic studies can be considered complete

In a study of the sources of infection the distribution of pneumococci in 180 normal individuals in Boston was investigated during the winter of 1923-24, these representing 26 families in whom there was no pneumonia

The results of this study are tabulated below

AGE	TYPE I	TYPE II	TYPE III	TYPE IV	NO PNEUMOCOCCI FOUND	PERCENTAGE OF CARRIERS
0-9	1	0	2	26	27	52
10-19	0	3	4	24	35	47
20-29	0	0	2	9	10	52
30-39	1	0	0	8	14	39
40-49	0	1	1	6	7	53
50 and over	0	0	0	3	2	60
Percentage of each type	1%	2%	5%	41%	51%	49%

In a similar study of 270 normal individuals in contact with 28 cases of lobar pneumonia the results are shown below. It is to be noted that all of the 28 cases of pneumonia were either Type I or Type III. Therefore, the occurrence of Type II in the exposed group would be expected to correspond with the occurrence of Type II in the nonexposed group—and this is the case

AGE OF CARRIER	TYPE I	TYPE II	TYPE III	TYPE IV	NO PNEUMOCOCCI FOUND	PERCENTAGE OF CARRIERS
0-9	2	0	2	17	47	41
10-19	2	1	3	15	14	60
20-29	2	0	5	14	37	36
30-39	4	2	3	14	22	51
40-49	0	1	3	11	19	44
50 and over	0	2	6	15	14	62
Percentage of each type	3.6%	2.2%	7.9%	31%	55%	45%

It is regarded as significant that the proportion of Types I and III is distinctly greater among those exposed to these cases

These studies show that there is no relation between the age of the contact and the distribution of the pneumococci. The degree of intimacy in the contact was of perceptible influence as might be expected

Virulence studies of pneumococci isolated from cases and carriers furnished some evidence that the former were rather consistently more virulent which is further borne out by evidence tending to show that contact with cases of pneumonia is more productive of positive contacts than is contact with healthy carriers

The inherent difficulties of the problem are shown by the fact that of fourteen healthy carriers in the control group not one gave a history revealing the source of the fixed type organism present

The study tends to indicate that cases of Type I and III pneumonia are more apt to produce carriers than carriers are apt to produce these types although carriers may produce homologous types of the disease in small numbers

As far as the evidence goes the disease seems mainly spread by contact

—R A K

Details

EVEN a casual glance at the Current Index of Medical Literature suffices to indicate the relatively enormous amount of information of varied kind—and value—which accumulates in the course of a single year and with which the alert physician, anxious to keep abreast of the newer developments, must have, at least, a speaking acquaintance

It is manifestly impossible for any one individual to familiarize himself with the innumerable minutiae concerned with medical progress and indeed it will be highly commendable if by effort and application he is enabled to keep in touch with the salient advances in the field in which his particular skill or interest lies. The important thing is to know where to find the information sought and how to apply it when found

With this end in view one is at times tempted to envy the verbal fecundity which produced the total ruin of the fabled jester of Rheims when, having sought and found a particular paper one finds that at least three or four readings coupled with much cogitation are necessary to excavate the particular point at issue

Certainly a writer describing a new method does so with the intention of making it available for the use of others, another presenting the conclusions formulated from his own experience or investigations behoves them worthy of consideration

Why not, then, present them in such fashion that others may repeat or utilize the work, or properly evaluate the conclusions drawn?

Perhaps this fault of lack of detail and clarity in the description of procedures is most common in the description of methods

It is unprofitable—and annoying—to read that so and so a method was employed when perhaps, the method has undergone several modifications and nothing is said as to which was employed or to say that such and such a technic was used when, as a matter of fact some modification of it was really used

It is somewhat difficult to attempt to try a new method for example, and to be confronted at the outset with loosely written reagent formulae

Attention has been called to this especially in connection with staining methods¹ in the description of which the following type of formula is frequently seen

¹Stain Technology April 1926 1 No 2 p 49

"Ale sol fuchsin	1 part
Water	10 parts"

What does this formula mean? What strength fuchsin? What strength alcohol? What fuchsin? Are the proportions measured by volume or weight?

How much more satisfactory to write

"Sat ale sol basic fuchsin (dye content 88%)	1 cc
Distilled water	10 cc"

Still more ambiguous and unsatisfactory are statements such as "Boil with dilute sulphuric acid" "Add a few drops of water" and so on

Examples in both clinical and technical papers could be cited almost ad infinitum

If it is worth saying at all it is worth saying well—and clearly!

—R A K

Errata

Article by Caven and Cantrow, October, 1926, page 76

Page 76, title should read A Method for the Determination of Calcium in Whole Blood

Third line, first paragraph, word *devised* should be *used*

The following paragraph should be substituted for the last paragraph on page 76 and the first paragraph on page 77

Following hemolysis and centrifugation a small precipitate is thrown down with the precipitate of calcium oxalate, which we believe to consist of the stroma of the disrupted red cells. Varying amounts of added calcium have been successfully recovered. Using this method values ranging from 6.5 to 9.5 mg per 100 cc of whole blood have been obtained. These figures are no doubt higher than the actual calcium content, probably due to the oxidation by the permanganate of some of the constituents of the stroma. This method was used to obtain a curve of changes in blood calcium following the injection of parathyroid extract, rather than to determine the absolute calcium content. It was found satisfactory for this purpose since any error caused by the constituents of the stroma should be practically constant.

Page 77, first reference, second line, the journal reference after the word *Report* should read, Jour Am Med Assn, 1926, LXXXVI, 1683, second reference, third line, journal reference should read, Arch Int Med, 1926, LXXXVIII, 502, third reference, fourth line, add p 461 to the reference

In the article "Studies in Toxicologic Chemistry I The Detection of the Opium Alkaloids by Selenious Sulphuric Acid The Specificity of the Reagent for the Phenolic Group" by Victor E Levine, June, 1926, issue, the fourteenth line from the top of page 811 should read Levine, 0.01 milligram of morphine equivalent to 0.025 milligram of morphine sulphate

The twelfth phenol in Table II, page 812, should have the following item

Dithionite	Purplish to cherry red in sulphuric acid alone,
(o hydroxybenzyl alcohol)	dark brown in selenious sulphuric acid

The Journal

of U. S. Department of Agriculture

Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., Editor in Chief
Medical Arts Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.B.
University of Toronto Toronto

Clinical Pathology

W. C. MacCARTY, M.D.
Mayo Clinic, Rochester Minn.

Biochemistry

VICTOR C. MYERS, Ph.D.
State University of Iowa Iowa City

Immunology

JOHN A. KOLMER, M.D.
University of Pennsylvania,
Philadelphia

Bacteriology

A. PARKER HITCHENS, M.D.
Bureau of Science Manila, P. I.

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

Pathology

PAUL G. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD B. WEBB, M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR, M.D.
Mayo Clinic Rochester Minn.

Experimental Medicine

RUSSELL L. HADEN, M.D.
University of Kansas Rosedale

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY 3523 25 PINE BLVD. ST. LOUIS.

Entered at the Post Office at St. Louis Mo. as Second Class Matter

LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment. Estimates Cheerfully Furnished. Prompt Service.

See our advertisements, pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA, PA.



KAHN STANDARD ANTIGEN

for

The Kahn Precipitation Test for Syphilis

Kahn Standard Antigen, Bio 104
is supplied in 10-cc vials

A complete direction circular
is enclosed in each package

LIST OF LABORATORY DIAGNOSTIC
AGENTS WILL BE SENT
ON REQUEST

Medical Research and Biological Laboratories

PARKE, DAVIS & COMPANY
DETROIT, MICHIGAN

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO JANUARY 1927

No 4

CLINICAL AND EXPERIMENTAL

EXSANGUINATION TRANSFUSION IN TREATMENT OF PHENOL POISONING*

BY CHAS C HASSELL, R C ALLEY AND P E PRILLAMAN
RICHMOND, VA

POISONING by phenol and the closely related cresols is still of such frequent occurrence as to render a knowledge of appropriate therapy most essential. When the poison has been taken orally gastric lavage is the most important measure to be employed, this being well illustrated by the statistics of Clarke and Brown.¹ These authors show that recovery almost invariably occurred when gastric lavage was practiced early even after doses as large as two ounces of liquefied phenol an amount over five times the estimated average fatal dose for man. Unfortunately, however, considerable time may elapse before the patient is seen by anyone familiar with the relatively simple technic of gastric lavage. It would be most desirable therefore, to secure a substance which is capable of detoxicating the phenol, either in the stomach or after it has been absorbed.

Of the various antidotes which have been proposed for phenol none has proved satisfactory. Some fifty years ago Baumann noted that phenol was excreted in the urine partly in the form of ethereal sulphates, from this observation the sulphates were suggested as phenol antidotes. As with the case of many other therapeutic measures whose only virtue is their novelty the sulphates were employed by certain investigators with apparent success in the treatment of phenol poisoning it was demonstrated by Tauher, and especially by Sollmann and Brown² that neither sulphates nor sulphites exercised any practical influence on the course of experimental phenol poisoning.

Apparently Seneca Powell was the first to suggest ethyl alcohol as an antidote for phenol.³ It was contended that the alcohol combined chemically

Received for publication June 18 1926
From Department of Pharmacology Medical College of Virginia.

with the phenol, thus detoxicating the poison. Gross explained the mechanism involved as consisting in the formation of "a new phenol benzene or aromatic compound" with the properties of ethyl alcohol. As in the case of the sulphates, this antidote, likewise, was used with apparent success in clinical poisoning by phenol, the observers disregarding the fact that gastric lavage was employed coincidentally in practically every case. In 1906, Clarke and Brown¹ reviewed the literature on clinical poisoning by phenol and carried out animal experiments, confirming the view that there was no chemical reaction between alcohol and phenol and demonstrating that alcohol possessed little superiority over plain water in gastric lavage.

Impressed, apparently, by the fact that a mixture of camphor and phenol is comparatively nonirritant, Wilson⁴ suggested that camphor was a valuable antidote for phenol. While camphor may serve to lessen the intensity of the local action of phenol, the two drugs have a central action not very dissimilar, consequently, it is not surprising to learn that Bond and Haag⁵ found camphor definitely to increase the toxicity of phenol.

The valuelessness of these supposed antidotes gives added interest to the paper of Robertson,⁶ in which is described a method for the treatment of various forms of systemic intoxication. Though primarily designed for combating the intoxication associated with severe burns, erysipelas, or acute intestinal disturbances in children, the author states that the application of this principle has been followed by success in cases of poisoning by carbon monoxide and by resorcin. Robertson terms his procedure "exsanguination transfusion", and states that it consists in the withdrawal of a large amount of blood and the introduction of an equivalent amount (or slightly more) from a healthy donor. In order to prevent collapse on the part of the patient as a result of the severe blood loss, the procedure is carried out by withdrawing only small quantities of blood at a time, and replacing this by the donor's blood, repeating as often as necessary.

A similar procedure was suggested in 1916 by Buemeister⁷ for the treatment of mercuric chloride poisoning. Buemeister removed moderate quantities of blood from the jugular vein of poisoned dogs and immediately injected a suspension of washed corpuscles. He was unable to save any of the treated dogs, but states that the intensity of the renal damage was less in these than in the controls. The use of Robertson's technic in the treatment of dogs poisoned by the intravenous injection of a just fatal dose of mercuric chloride has likewise proved incapable of preventing a fatal outcome in our experience⁸, nevertheless, because of the favorable report of Graham and Tisdall⁹ on the use of transfusion or of bleeding and transfusion in the treatment of resorcin poisoning, it was felt that the value of the procedure in phenol poisoning should be made the subject of investigation.

Bond and Haag⁵ place the fatal oral dose of liquefied phenol for dogs at about 0.4 cc per kilogram body weight. Since they administered this amount to only three dogs, it was determined to run a larger series of animals in confirmation of their results.

The dogs used in our experiments were full-grown, apparently healthy adults. They were fasted twenty-four hours, but were allowed free access

to water at all times Morphine sulphate, 20 mg per kilogram, was injected subcutaneously, and, in about thirty minutes, the phenol was given through a stomach tube Undiluted liquefied phenol was employed this was measured from a burette into a small funnel connected with the stomach tube and was followed by from 50 to 150 cc of wash water The results of these experiments are given in Table I

TABLE I
RESULTS OF ORAL ADMINISTRATION OF PHENOL TO DOGS

DATE	WEIGHT IN KG	MORPHINE MG X KG	PHENOL CC X KG	RESULT
3-26-25	7.7	20	0.3	Died in 3 days.
3-30-25	1.7	20	0.3	Recovered
4-3-25	14.0	20	0.3	Recovered.
4-4-25	19.4	20	0.3	Died in 2 days
4-6-25	10.6	20	0.4	Died in 2 days.
4-7-25	6.5	20	0.4	Died in 24 hours
4-10-25	11.5	20	0.4	Died in 2 days
4-15-25	9.9	20	0.4	Died in 6 days
5-4-26	15.9	20	0.4	Died in 24 hours
5-4-26	15.0	20	0.4	Died in 2 days
5-4-26	15.7	20	0.4	Died in 24 hours.
5-4-26	13.4	20	0.4	Died in 24 hours
5-4-26	11.9	20	0.4	Died in 5 days
5-4-26	11.6	20	0.4	Died in 24 hours.

From these results, it was assumed that the fatal dose of liquefied phenol administered to dogs in the manner described lay between 0.3 and 0.4 cc per kilogram body weight

The dogs treated by exsanguination transfusion were first given morphine sulphate, subcutaneously, 20 mg per kilogram and then the liquefied phenol orally Within a few minutes the characteristic action of the phenol became manifest the animals losing consciousness and developing coarse convulsive twitching of the striated muscles After varying lapses of time the femoral vessels were exposed the depression of the central nervous system by the phenol being so great as to render the use of any other anesthetic unnecessary Blood was withdrawn from the artery in an amount varying with the weight of the dogs and this was followed immediately by injection of a slightly greater amount of citrated blood from an apparently healthy donor into the femoral vein The blood of the donor had previously been matched with that of the recipient Only three animals were treated in this way the results were so uniformly unfavorable as to discourage further efforts along this line These results are given in Table II Here, as in the remaining tables, when an animal died during the night subsequent to administration of the phenol its death was said to have occurred "in twenty four hours" In many, indeed most instances the duration of life was considerably less than twenty four hours in view of our ignorance of the exact time of death, the rule mentioned was arbitrarily adopted

Reference to Table I shows that of four control dogs given the oral dose of 0.3 cc liquefied phenol per kilogram body weight, two recovered without treatment Both the animals that received this dose and were treated by exsanguination transfusion not only succumbed but death occurred much

TABLE II

RESULTS OF EXSANGUINATION TRANSFUSION TREATMENT OF PHENOL POISONING

WT IN KG	PHENOL CC X KG	TIME	BLOOD DRAWN	TIME	BLOOD INJECTED	TIME	RESULT
5.0	0.3	5 10	150 cc	5 42	175 cc	5 53	Death 15 min
3.5	0.3	5 35	100 cc	6 12	150 cc	6 25	Death 3 min
5.8	0.4	3 35	175 cc	4 20	145 cc	4 30	Death 24 hrs

more promptly than in the case of any of the control dogs. Strangely, the dog that was given 0.4 cc of phenol and then treated by exsanguination-transfusion lived longer than either of the animals receiving the smaller dose of phenol. It is to be noted that this dog received less blood than was removed from its vessels.

Similar unfavorable results were observed in a larger series of animals given barely fatal doses of mercuric chloride intravenously and then subjected to the exsanguination-transfusion therapy.³ In these animals, the technique of Robertson was strictly adhered to: relatively small amounts of blood were withdrawn repeatedly, to be promptly replaced by injections of blood from the donor. Although the bloods of the donor and recipient were roughly matched before the administration of the phenol, the suspicion arose, naturally, that either the bleeding or the subsequent injection of the citrated blood was responsible for the unfavorable results. In order to determine the influence of the bleeding alone, a series of nine dogs was used. These animals were given morphine and varying doses of phenol, the procedure being similar to that used in the previously described experiments. In from twenty-three to seventy minutes, the dogs were bled from the femoral artery and then given intravenous injections of isotonic salt solution, the volume of the latter being slightly greater than that of the blood withdrawn. The outcome of these experiments is given in Table III.

TABLE III

RESULTS OF EXSANGUINATION AND SALINE TRANSFUSION TREATMENT OF PHENOL POISONING

WT IN KG	PHENOL CC X KG	TIME	CC BLOOD DRAWN	TIME	CC SALINE INJECTED	TIME	RESULT
15.4	0.3	3 26	470	4 10	500	4 20	Recovery
12.5	0.4	4 31	375	5 10	435	5 20	Recovery
11.7	0.4	3 45	351	4 55	400	5 00	Recovery
10.0	0.4	3 40	300	4 18	350	4 30	Death 24 hours
12.0	0.4	4 58	360	5 30	420	5 38	Death 24 hours
8.9	0.4	4 10	280	4 45	320	4 55	Death 24 hours
15.9	0.4	12 02	500	12 25	600	12 31	Death 24 hours
13.4	0.4	12 34	400	1 38	600	1 39	Death 6 hours
15.6	0.4	12 39	470	1 02	650	1 12	Death 24 hours

The fact that two of the dogs that were given the dose of 0.4 cc of liquefied phenol per kilogram body weight recovered after bleeding and saline injection would appear to indicate that this procedure possesses some merit in the treatment of phenol poisoning. Opposed to this, however, it is apparent that death occurred more quickly in the fatal cases of this series than in the series of control dogs, the average duration of life after adminis-

tration of phenol in the former being less than twenty four hours, in the latter, about two days. It is certainly safe to conclude, however, that with drawing large amounts of blood and subsequently injecting isotonic saline has no such detrimental effect as seen when the bleeding was followed by the injection of citrated blood.

In spite of the fact, previously mentioned, that the bloods of donor and recipient were roughly matched before transfusion, the conclusion could scarcely be escaped that the unfavorable results were connected with the injection of the blood. The next step therefore, was to determine the effect of exsanguination transfusion on unpoisoned dogs. In order to do this, seven dogs were used. The animals were given 20 mg of morphine sulphate per kilogram subcutaneously, and, under ether anesthesia, the femoral vessels were exposed. Amounts of blood equivalent to from 3 to 5 per cent of the body weight were withdrawn from the artery, and this was followed immediately by the injection of citrated blood into the vein. In two cases, the operative wound became badly infected, the dogs succumbing, apparently to this infection, on the fourth and seventh days respectively. A third dog seemed to be doing well but suffered a fatal hemorrhage on the fifth day. The remaining dogs seemed uninjured by the procedure, and were apparently normal in from eight to eleven days, when they were used for other purposes.

CONCLUSIONS

The following conclusions seem justified

- 1 The minimal fatal dose of liquefied phenol by oral administration to dogs that have been previously fasted twenty four hours and given morphine to prevent emesis is in the neighborhood of 0.3 to 0.4 cc per kilogram body weight.
- 2 The withdrawal of large amounts of blood and the subsequent injection of presumably compatible citrated blood exerts a definitely unfavorable influence in poisoning by phenol. The reason for this is obscure.
- 3 The withdrawal of large amounts of blood and the subsequent injection of isotonic saline may be of some benefit in the treatment of phenol poisoning, if the procedure is of any value. It is slight.
- 4 Exsanguination transfusion except in so far as it may offer a portal of entry for infection or give rise to the possibility of hemorrhage appears to be a safe procedure for unpoisoned dogs.

REFERENCES

- ¹Clarke and Brown. The Value of Alcohol in Carbolic Acid Poisoning, Jour Am Med Assn, March 17 1906, xlv, 782.
- ²Cited by Sollmann and Brown. Jour Am Med Assn, March 23 1907, xlviii, 1015.
- ³Sollmann and Brown. The Value of Sulphates in Carbolic Acid Poisoning. Jour Am Med Assn, March 23 1907, xlviii, 1015.
- ⁴Wilson. Practical Druggist, May 1924.
- ⁵Road W. R., and Haag H. B. The Synergistic Action of Camphor in Phenol Poisoning. Jour Am Pharm Assn, February 1925, xiv.
- ⁶Robertson L. B. Exsanguination Transfusion. A New Therapeutic Measure in the Treatment of Severe Toxicosis. Arch Surgery, July 1924, lx, 1.

- ⁷Burmeister, W H The Effect of Extensive Venesection and Transfusion on Kidney Lesions in Severe Acute Mercuric Chloride Poisoning, *JOUR LAB AND CLIN MED*, 1916-1917, II, 500
- ⁸Haskell, Chas C, Hamilton, J R, and Henderson, W C Exsanguination Transfusion in the Treatment of Mercuric Chloride Poisoning, *JOUR LAB AND CLIN MED*, May, 1926, XI, 707
- ⁹Graham and Tisdall Poisoning from the External Use of Resorcin, *Can Med Assn Jour*, N S, October, 1922, XII, 730

A CONTRIBUTION TO THE NATURE OF DIABETES*

A MATHEMATICAL DERIVATION OF THE BLOOD-GLUCOSE CURVE

BY DWIGHT M ERVIN, MD, SAN FRANCISCO, CALIF

IN A previous article it was stated that failure of the oxidation of glucose could not be the basis of the condition of diabetes¹ This belief has led to the mathematical construction of the blood-glucose curve in order to see what effect the failure to oxidize glucose would have upon the normal curve

The glucose tolerance curve as generally given is a curve resulting from the rate that glucose is absorbed from the intestinal tract, the rate the glucose is converted into glycogen, and the rate that the glucose is oxidized from that absorbed There is a definite mathematical rate that glucose will be absorbed from the intestinal tract from a given concentration Likewise the rate that glucose will be converted into glycogen depends upon the concentration of the glucose in the blood stream The rate of oxidation, however, is independent of both the intestinal tract and blood concentrations

When once the blood-glucose tolerance curve is obtained in mathematical form it is possible to investigate each rate individually and its effect upon the curve when the rate is altered If we choose to investigate the effect of oxidation upon the curve we may do so by changing the rate of the oxidation as is presumably done in the diabetic We may even decrease the rate of oxidation to zero, making a complete diabetic, and investigate the effect of this zero oxidation upon the curve

As glucose is absorbed from the intestinal tract its rate at any point of time in the entire length of time of the absorption is dependent upon the concentration of glucose in the intestinal tract at that point of time But this concentration is always decreasing by the amount absorbed

Let a = initial concentration

x = quantity absorbed

dx

$\frac{dx}{dt}$ = rate of absorption

dt

(1) $\frac{dx}{dt}$

$= k_1 (a-x)$

k_1 = constant of absorption

do

$\frac{do}{dt}$ = rate of oxidation which is constant

dt

$$(2) \quad \frac{da}{dt} = c$$

$c = \text{constant of oxidation}$
 Integration of equation one gives--
 $a - a_0 = -k_1 t$ $= x$ quantity absorbed
 integration of 2
 (3) $a = ct$ quantity oxidized at any given time

Hence the quantity in the blood stream at any given time if glycogen formation does not take place is the quantity absorbed less the quantity oxidized

$$(4) \quad a - a_0 e^{-k_1 t} - ct = \text{the blood stream concentration}$$

As the glucose is absorbed the conversion into glycogen takes place and the rate that it does so will depend upon the glucose passing into the blood stream less the quantity converted into glycogen and oxidized at any point of time

$$(5) \quad \frac{dz}{dt} = k_2 y$$

where $y = \text{the quantity in the blood stream}$
 $k_2 = \text{constant of glycogen formation}$

$$(6) \quad \frac{dy}{dt} = \frac{dx}{dt} - \frac{dz}{dt} - \frac{da}{dt}$$

Substituting the values for $\frac{dx}{dt}$, $\frac{dz}{dt}$ and $\frac{da}{dt}$ and integrating the equation we derive the value of the quantity of glucose in the blood at any point of time

$$(7) \quad y = \frac{k_1 a_0}{k - k_1} e^{-k_1 t} - \frac{k_2 a_0}{k - k_1} e^{-k t} - c t e^{-k t}$$

The test of this equation must be that as k (or the rate of glycogen formation) becomes zero the equation must equal the amount absorbed less the amount oxidized

$$\text{making } k_2 = 0$$

$$7 \text{ becomes } y = a - a_0 e^{-k_1 t} - ct = \text{equation 4 or } x - ct$$

In this equation the rate of oxidation is independent of the concentration in the blood stream. This is not strictly true for Lusk found the specific dynamic action for glucose to be about 10 per cent. For this purpose the increase of oxidation is very small and will not affect the curve within the limits of practical errors.

There are to this equation of glucose in the blood stream three constants, k_1 , k , and c . k_1 determines the rate of absorption k the rate of glycogen formation and c the rate of oxidation.

The values of k_1 , k , and c may be determined as follows. For any given case that is normal we may obtain two important points. The crest of the

blood-sugar curve and the quantity in the blood at an interval of three hours. To an individual of 70 kilos weight, 95 gm of glucose were given in one liter of water. At 0.7 hours the crest of the curve was reached and at 3.0 hours the curve had returned to 30 mg above normal.

Seventy kilos weight gives closely 5 kilos of blood. When 100 mg are present per 100 c.c. of blood there is a total of 5 gm. When 35 mg are present there is a total of 1.7 gm.

At the crest of the curve the tangent to the curve is equal to zero.

$$(8) \quad \frac{dy}{dt} = 0 \quad (t = 0.7)$$

Differentiating $7k_2 = nk$ and setting it equal to zero we have

$$(9) \quad 0 = -\frac{k_1 a e^{-k_1 t}}{n-1} + \frac{k_1 a e^{-k_2 t}}{n-1} - (c - e k_2) e^{-k_2 t}$$

At t equal 0.7 the blood glucose has risen from 100 mg at the beginning of the test to 200 mg. This is equal to an increase of 5 gm.

Substituting $a e^{-k_2 t}$ from $7 \quad (t = 0.7, y = 5)$ and solving for n we get
 $n = 1$ or 13
 $k_2 = 1k$, or $13k$,
 again from $7 \quad (t = 3, y = 1.7)$
 $k_1 = 0.38$
 $k_2 = 4.94$

The computation of c is much simpler. From the basal metabolism at the respiratory quotient of 0.84 we find 45.6 per cent of the calories are derived from glucose.

Height 70 inches

Weight 70 kilos

Total surface 1.78 square meters

Total metabolism per hour 712 calories

Calories derived from glucose per hour 324

Grams of glucose burned per hour 8.0

Constant $c = 8.0$

In Chart I, Curve 1 is the blood curve from which the equation was derived. Curve 2 is the curve of the derived equation.

With the equation of the curve developed we can investigate each of its parts that enter into its structure. If we desire to find how the normal curve would be altered when we decrease the rate of oxidation in the body we have but to decrease the value of the constant c .

Let c be made zero in equation 4 and the equation now becomes—

$$y = \frac{k_1 a e^{-k_1 t}}{k_2 - k_1} - \frac{k_1 a e^{-k_2 t}}{k_2 - k_1} \quad \text{the equation of a complete diabetic}$$

If this curve is now plotted as the curve that a diabetic would develop if he did not oxidize sugar—a complete diabetic—we find it even in the most

exaggerated form to differ only in the smallest degree from the normal. From a mathematical point of view, if we grant that absorption and glycogen formation are consecutive simultaneous reactions, it is impossible for the failure of oxidation to produce the curve that is found in the diabetic. If the diabetic does not burn glucose it is a fact unrelated to his blood glucose curve.

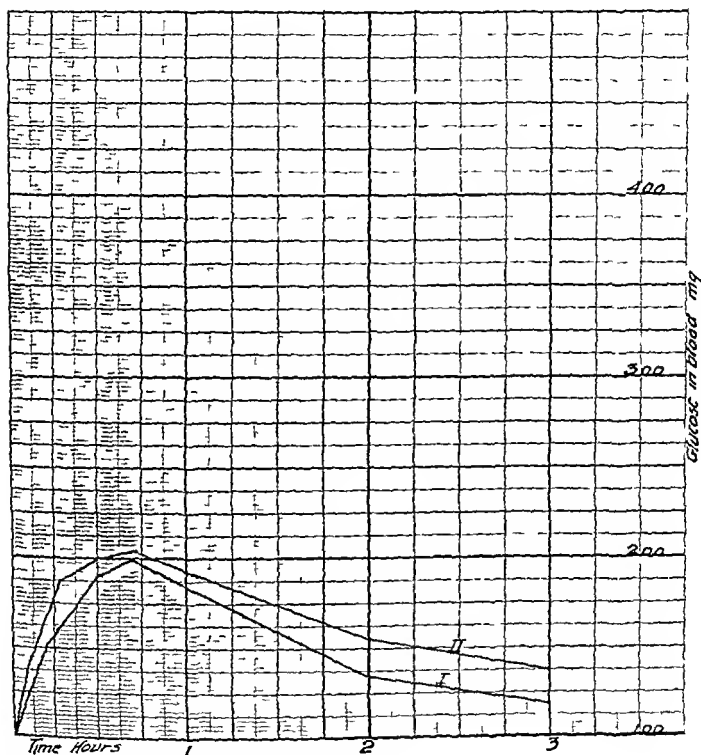


CHART I

and nothing concerning this failure to burn glucose can be derived from the blood glucose curve. Chart II, Curve 1, normal, Curve 2, oxidation = zero.

We may investigate the remaining constants or rates of reactions, absorptions and glycogen formation. k_1 , or the constant of absorption is not likely the cause of the running of glucose in the urine. If it should be the cause it would have to be tremendously increased in value to cause the diabetic curve.

The constant of the rate of glycogen formation, or k_2 , may next be altered and the effect upon the curve of a decreased rate of glycogen formation be found. If we make the rate of glycogen formation equal to zero, equation 4 becomes—

$y = a - ae^{-k_1 t} - ct$ or the quantity of glucose absorbed less the quantity burned. Chart III is a comparison of $k_2 = 0$ or a complete diabetic if dia-

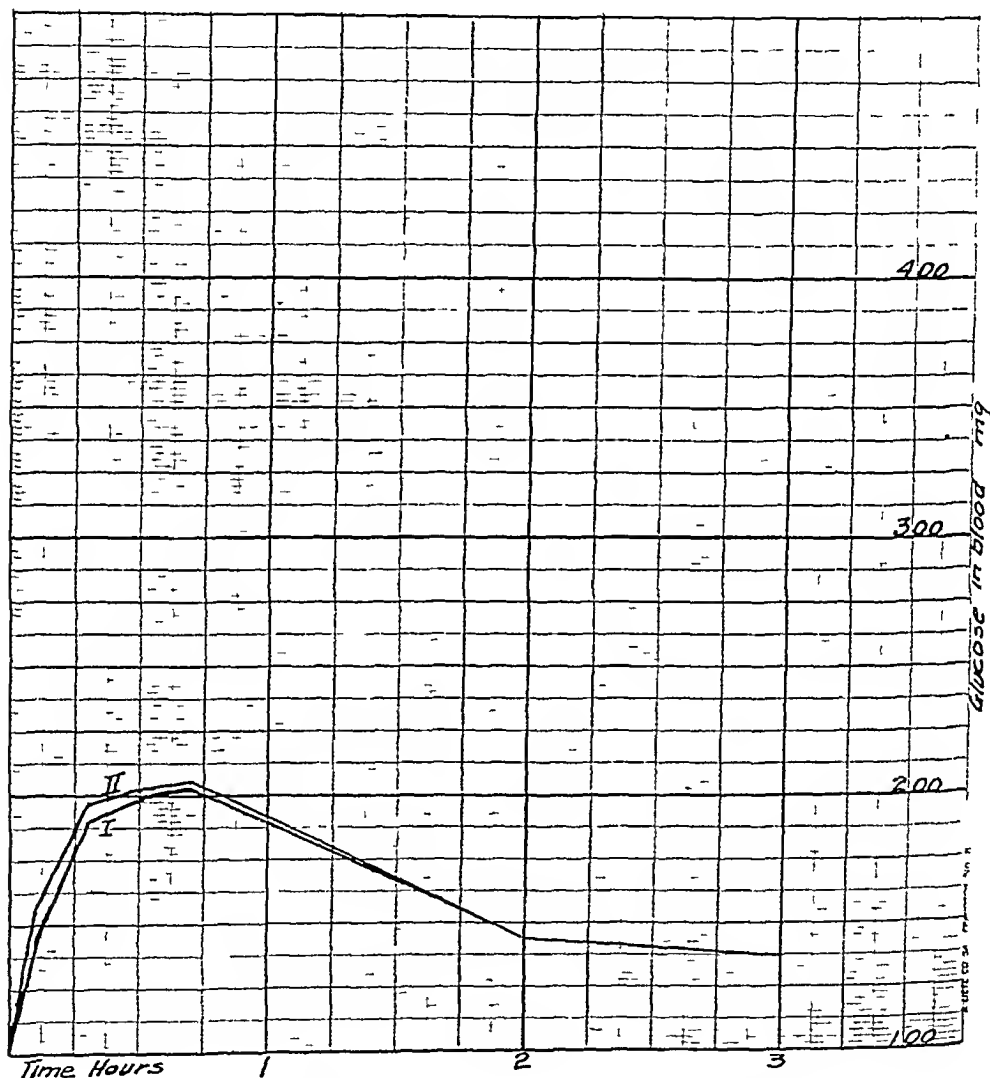


CHART II $c=0$ Curve II curve I = normal

betes is the failure to, or decrease in rate of the formation of glycogen. Since if the blood volume is 5 liters and the glucose is given in one liter of water diffusion would take place until $\frac{1}{5}a$ had diffused. The quantity $ae^{-k_1 t}$ is the quantity of glucose left in the intestinal tract. The value of y would cease to rise when $ae^{-k_1 t} = \frac{1}{5}a$ or $y = \frac{4}{5}a - \frac{1}{5}ae^{-k_1 t} - ct$.

Chart III includes this correction for the quantity of glucose that diffuses. The alteration of k_2 changes the normal into a curve that approaches that of the diabetic, the sharper rise, the greater height, the delayed, longer, flatter crest, and the slower decline to the normal. We may give k any value we choose between $k = \text{normal } (4.94)$ and $k_2 = 0$

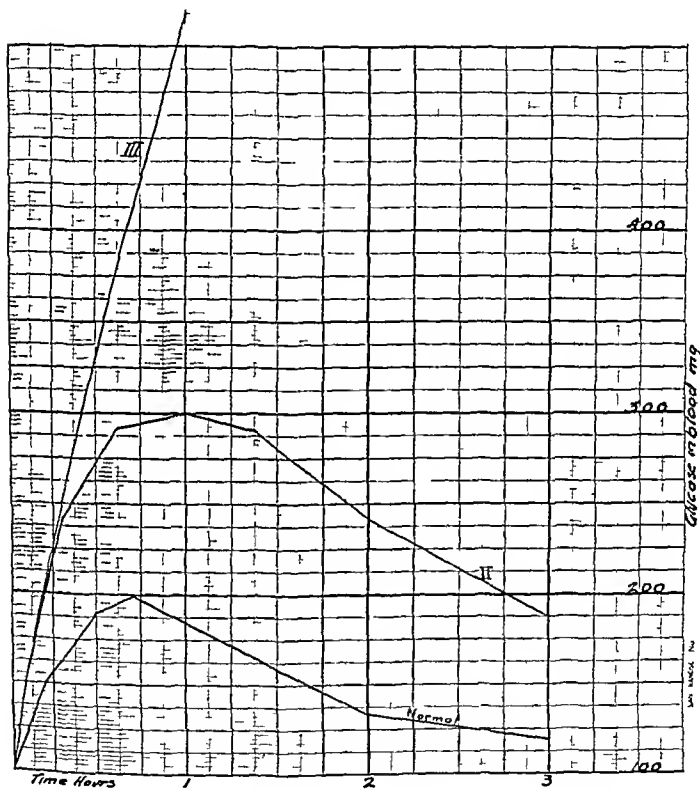


CHART III curve II - $k_2 = \frac{1}{2}$ normal curve III $k_2 = 0$

Chart III gives the curve when the rate of glycogen formation is one half the normal $k_2 = 2.60$. Such a curve is typical of what we find in the diabetic, and is only different in that above the kidney level the curve is altered by the rate of kidney secretion. Such a curve including with this curve the rate of kidney secretion will be published in a future paper.

The slope of the rise, the height of the crest, the sharpness of the crest,

the early or late crest are all dependent upon the values and ratio of k_1 and k_2

If the ratio $\frac{k_1}{k_2}$ or $k_2 = nk_1$ is kept constant the crest may be made earlier or later by decreasing or increasing the values of both. Hence an early or late crest without the height being considered has no value in the interpretation of a curve. No single feature of the curve taken by itself will give a satisfactory interpretation. All should be considered. The only single feature of the curve that yields a knowledge of what is going on in the curve is the ratio $\frac{k_1}{k_2}$ and an idea of this may be gained from the rate of change of the tangent

If we inspect the curve of the normal at 0.7 hour we find the tangent at this point parallel to the time ordinate, but in Curve 3 of the diabetic the tangent at 0.7 hour has not yet become parallel to the time ordinate. The rate of change of the tangent is indicative of the ratio $\frac{k_1}{k_2}$

The tangent at any point on the curve is equal

$$\text{Tan} = \frac{dy}{dt}$$

$$\text{Tan} = -\frac{k_1 a e^{-k_1 t}}{n-1} + \frac{k_2 a e^{-k_2 t}}{n-1} - (c - c k_2) e^{-k_2 t}$$

Hence $\text{Tan}(t-2) - \text{Tan}(-3)$ is greater in the normal than in the diabetic. Above the kidney level the values of the curve are misleading.

Since the crest of the curve of a normal is close to 0.7 hour and the diabetic of $\frac{k_2}{2}$ crosses the kidney level at about 0.2 hours there is in the glucose curve a very small range in which we may work.

For this reason a starch curve where k_1 is decreased by the delayed absorption due to the digestion is far better for interpretation of the degree of diabetes. k_1 for 20 gm of starch per kilo weight is at present being determined for the normal.

In the attempt to test our old work¹ by the mathematical equation of the blood-glucose curve we find ourselves back to old position upon the nature of diabetes. The failure to burn glucose can have nothing to do with the presence of glucose in the urine. The glucose curve and the glucose in the urine are not factors of oxidation but of the velocity of glycogen formation or the constant k_2 . This leaves only the problem of the "acetone bodies" to the theory of oxidation or failure to oxidize in diabetes, and work in preparation now shows that the formation of the acetone bodies may be accounted for independent of the oxidation of glucose.

As more cases are worked the values of k_1 and k_2 may be determined over a wider range of normals. From this curve the so called adrenal and thyroid effects can be unravelled by determining which constant, if any, is affected.

SUMMARY

A mathematical equation for the blood glucose curve of the blood is derived from the three factors, absorption from the intestinal tract, formation of glycogen, and oxidation.

The constants of the curve determining the rate that absorption, glycogen formation, and oxidation goes on are determined.

From these constants the effect on each function on the curve can be investigated.

By placing the constant $c = 0$ we make the rate of oxidation equal to zero—or if diabetes has to do with the burning of glucose—we make a complete diabetes.

The curve derived from failure to burn glucose differs only insignificantly from the normal and in no way approaches the curve found in diabetes.

By investigating mathematically the condition of diabetes from the blood glucose curve no evidence of the failure to burn glucose can be obtained. But rather that the curve depends only upon a decreased rate of glycogen formation.

REFERENCE

LEVIN, D. M. JOUR. LAB. AND CLIN. MED., 1919. v

ISOPROPYL ALCOHOL—AN INVESTIGATION OF ITS PHYSIOLOGIC PROPERTIES²

By HENRY C FULLER, B S, AND OSCAR B HUNTER, A M, M D
WASHINGTON, D C

THE investigations herein reported were instituted in 1921 and comprised a study of the effect on the animal economy of isopropyl alcohol (isopropanol) administered in such dilutions that the acute or local action on the membranes and tissues would be negligible. In other words, it was desired to obtain data on the general systemic action of the substance.

Coincident with the physiologic investigation on animals a series of tests was performed to determine the effect of isopropyl alcohol on bacteria, yeasts and molds in comparison with ethyl alcohol.

PHYSIOLOGIC ACTION

The work included not only a study of the effects of isopropanol on the animal economy, but a comparison of its physiologic action with that of ethyl or grain alcohol, and its effect on bacteria and molds. Its action on the animal economy was determined by observing the effects of its administration to living animals and human subjects. The animals employed included rabbits, dogs, cats, guinea pigs, chickens and a monkey.

In order to give some idea of the way the work was performed the investigation will be briefly outlined.

Subjects—As noted above, the subjects selected included rabbits, guinea pigs, dogs, cats, chickens, monkeys and humans. Careful attention was given to their health and none but sound vigorous adult specimens were employed. The rabbits were large gray specimens of the Belgian hare type, averaging in weight about four pounds. The animals were kept in a large airy room under normal temperature conditions and were plentifully supplied with water and good wholesome food. The cages were kept in as sanitary a condition as possible, one attendant devoting a considerable part of his time to keeping them clean and attending to the wants of the occupants.

Observations on Living Animals—Each animal was kept under observation for a period of a week or two before submitting it to the tests. In this way it became possible to observe the normal behavior of the animal, its preferences as regards food and its individual eccentricities if it possessed any.

Careful observations of its daily fluctuations in weight were recorded, the character and frequency of its eliminations, and its general deportment, nervousness, reaction to human contact, etc.

²Received for publication July 14 1926

At the end of its probationary period, it was given a clinical card and admitted to the list of animals receiving the test material

While under treatment a careful record was kept of its weight, desire for food, condition of its pelage or plumage, and all symptoms of a pathologic nature due directly to the substance administered

The observations and method of handling the human subjects will be described later

Observations After Death—Several animals died during the course of the investigation through accident or through contraction of disease (usually pneumonia) In nearly all cases those which came through the tests alive were killed, though in certain exceptional instances animals were allowed to survive for a considerable period after the conclusion of the tests in order that special observations might be recorded These were subsequently killed In every instance, no matter how the animal came by its death, it was autopsied, the gross pathology noted and then the various organs were immersed in formaldehyde solution These specimens were preserved in separate jars, numbered to correspond with the clinical card of the subject, and later sectioned and examined microscopically to determine the pathologic conditions

Technic—The isopropanol was mixed with an equal volume of water for administration Doses of not less than 5 cc nor more than 20 cc were given Ingestion was effected by introducing a soft catheter through the mouth into the stomach of the animal and then gently forcing the liquid through the tube by means of a syringe attached to the open end The syringe was of glass, with a graduated barrel After receiving the dose the catheter was withdrawn and the animal returned to its cage or placed on an observation table

The catheter was not employed in administering the liquid to guinea pigs These were restrained by hand the mouth opened and the liquid introduced with a medicine dropper whereupon the animal immediately swallowed the dose

The details of the work were administered by a chemist a pathologist and an ophthalmologist

General Observations—In running the tests with isopropanol careful comparisons were made with the effect of ethyl alcohol administered under identical conditions With some subjects these substances were administered alternately that is, one day the rabbit or chicken received a dose of isopropanol, and the next day ethyl alcohol By this means the individual susceptibility was determined and a better comparison of the reactions could be gained

In every instance it was noted that at first the animal reacted more sharply to the isopropanol than it did to the ethyl alcohol The first two or three doses of isopropanol might cause complete inertia and prostration, lasting sometimes for several hours while with equivalent doses of ethyl alcohol, though there was usually a marked incoordination, coma never resulted

It was interesting to note however, that after two or three administrations, the animals acquired a tolerance to isopropanol, and thereafter they reacted no more seriously than they did to ethyl alcohol. In one case, notably a chicken, the first dose of 20 cc of 50 per cent isopropanol produced a condition of collapse, lasting thirty-six hours. Recovery was complete, however, and the bird subsequently developed such a tolerance that she could ingest a similar quantity with no more effect than a well-defined incoordination of the legs. This subject received 280 cc of 50 per cent isopropanol between October 20 and November 30, 1921. During the observation she lost weight and had an unhealthy appearance, but on terminating the dosages, she recovered her health and apparently became normal with brilliant comb and wattles, and sleek plumage.

The effect of isopropanol on cats was extreme and almost immediate. They lost control of their hind legs and after floundering about for a short time, passed into a stupor from which they could not be aroused. After several hours they recovered and from that time, were apparently normal.

With dogs, the effect was delayed and these animals were much less susceptible to its effects than were cats. Incoordination was apparent, but they never exhibited the absolute stupor shown by the cats or the prostration that was rendered by the first few doses on rabbits.

The observations made on guinea pigs were not satisfactory. These creatures are small, and the individual susceptibility of the test animals varied to such an extent that it was difficult to control the dosage, and at the same time determine whether the reactions observed were due to an overdose of the product or to the sensitiveness of the animal.

Gross Pathology—Most of the rabbits showed chronic passive congestion of the stomach, intestines, liver or kidneys, one rabbit showing the effects in one of these organs, another with a different organ. In some cases, inflammation of the stomach and intestines was also evident. These conditions occurred in both the isopropanol and ethyl alcohol subjects.

It will be noted from the detail summary below, that some of the subjects ingested a considerable bulk of isopropanol during the course of the investigation. It is not surprising, therefore, that chronic gastric pathogenesis was established. It would have been more remarkable had the introduction of a substance so foreign to the normal diet of the creature not set up some disturbance of the alimentary tract, or of the vital organs.

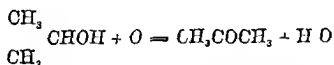
In some cases, blurring and "cupping" of the optical discs was noted, but this condition was found equally marked in case of the ethyl alcohol rabbits. The changes noted in the optical system are not of great moment and it should be emphasized that the effects of isopropanol in this respect are no more momentous than are those of ethyl alcohol. No evidence of blindness was apparent in any instance. It must be borne in mind, however, that the optical system of the rabbit differs in certain particulars from that of the higher animals. For this reason, careful special observations were made of the action of isopropanol on the optic nerve and the eye of the monkey and human being. The result of this last series of tests, to be later detailed, showed that not only was there no impairment of vision or other observable

effect, but in the case of the human subjects most of them reported that during the time of the trial, their ability to use their eyes was noticeably enhanced.

During the course of the work, careful observations were made of the condition of the urine with respect to the presence of acetone. On account of the quantity voided and the facilities attendant to its collection, only that of the dogs and human subjects was examined. It was found that the ingestion of isopropanol produced acetone in the system, the same being eliminated through the kidneys and found in the urine by chemical test. It appeared within twenty-four hours after ingestion and was constantly present until the isopropanol was withdrawn, after which time no more was noted.

The appearance of acetone in the urine was at first the occasion of some concern, inasmuch as its presence is ordinarily an indication of a metabolic disturbance in the system, due to the breaking down of the fatty tissues. However, the reason for its presence in this case is obvious when the character of isopropanol is taken into consideration. It is well known to the physiologist that alcohols are oxidized in the body. And it is equally a matter of common knowledge to the chemist that primary alcohols, such as ethyl alcohol, yield aldehydes on oxidation but that secondary alcohols do not; they are decomposed with the production of ketones and if one glances at the structure of isopropanol (isopropyl alcohol) as noted herewith

CH_3
 CHOH , it is apparent at once that the products of its oxidation must be CH_3
 acetone and water.



The fact must be borne in mind that the presence of acetone in the urine of diabetics is not the cause for concern for the subject. The acetone is simply an indicator of a metabolic change that is taking place and is a constant factor in the course of the disease. In the matter under discussion, the acetone is produced by an entirely different action going on in the system, and its presence ceases as soon as the ingestion is terminated.

Fate of Control—During the entire course of the rabbit tests, a control animal was kept under observation exposed to the same condition of temperature and environment and fed with the same ration. The record began on September 20, 1921, at which time the animal weighed 2150 gm and was in apparent good health. Its behavior throughout the period of observation was normal. On November 25, 1921, it was killed and autopsied, weighing on that date 2225 gm. The examination showed some coccidiosis but otherwise the organs were normal.

It will be noted that this rabbit showed coccidiosis and that the same condition was observed in many of the rabbits used for these tests. For the benefit of the reader who may be unfamiliar with medical terms it should be stated that coccidiosis is a condition of the liver usually peculiar

to rabbits, manifested by a pustular eruption. It occurs in rabbits that are kept under normal conditions just as frequently as it does in those being used for experimental purposes, and its presence is not a factor in judging the good or bad effects of the product under discussion.

TESTS ON MONKEY AND HUMAN BEINGS

Monkey—The monkey selected was a white-faced capucin, not too small, but of such a size that restraint could be maintained without undue force, as the monkey is a very difficult animal to handle, and in its struggles may become more seriously injured by overexertion or mechanical contact, than by the test material. He weighed four pounds.

For this experiment the animal was kept under careful observation for two weeks until it became accustomed to the surroundings and the attendants, and was eating regularly. Restraint was maintained by holding the monkey in the hands protected by automobile gauntlets, two other attendants holding the hands and tail. The monkey soon became accustomed to swallowing the catheter and after a few days of struggling, submitted to the experiment with comparative ease in handling.

Doses of 5 c.c. of 50 per cent isopropanol were administered.

On January 19, 1922, the animal was given a dose in the amount above noted and soon developed mild symptoms of intoxication, followed by drowsiness and the indisposition to eat. This test was repeated November 23, with similar results. On the following day he was given 10 c.c. or double the quantity previously administered with the result that definite symptoms of intoxication became manifest, muscular incoordination, nausea, vomiting, extreme lassitude and indisposition to move occurred, lasting for about twenty-four hours. The animal was allowed to recover completely before further administrations.

Beginning January 27 and continuing until February 14, or thirteen days during the interval, doses of 5 c.c. were given. In every instance, the symptoms produced were mild and the animal had apparently established a considerable tolerance to the isopropanol.

The administrations were terminated February 14, and the monkey kept under clinical observation until June 29 when he was transferred to a large cage at the National Zoological Park. He was still alive on December 14. He has since died. In appearance he was greatly inferior to the other capucins, was thin and apparently in poor health, though he ate well.

The conclusions of the pathologist may be summarized as follows. The symptoms produced on the monkey are not unlike those produced by ethyl alcohol. Definite intoxication by isopropanol is seen. Gastroenteritis, with indisposition to eat, is met with in the case of ethyl alcohol as well as with isopropanol.

After each administration the animal was always more or less affected but to a much less degree of intensity in the later stages of the experiment than in the earlier. He would often lie in a corner of the cage for a while after receiving the dose, but his eyes were not always closed and consciousness of outside happenings was usually manifested.

The appetite was much affected, food being relished with less enthusiasm than before the period began.

As this experiment had for its special object, the study of the action of isopropanol on the optical system, the observations of the oculist are noted in detail.

Prior to the inauguration of the tests, it was observed that the right optic disc was somewhat more engorged than the left but the eyes were otherwise normal. After the first ingestion, the examination showed both optic discs congested but otherwise the eyes were normal. Subsequent examinations were virtually the same, always more or less congestive.

March 2, two weeks and a half after the test was terminated, the oculist reported that both eyes were apparently normal. The pathologist therefore asserts that isopropanol in the dosage given produced some congestive disturbances on the eyes, but no organic changes that would result in blindness.

Human Beings—As the experiments thus far conducted had indicated that isopropanol was a substance of relatively low toxicity, and as it apparently had no effect on the optical system, it was decided expedient to note the reaction of human beings to its ingestion.

Grant and Johns of the Standard Oil Company in articles published in the *American Perfumer*, 1921, and the *American Journal of Pharmacy*, 1921, referred to Sinclair's tests of the local effects of isopropyl alcohol (isopropanol) on the skin of human beings, the conclusions of the authors being that it was without harmful action.

For the experiments to be described there were selected healthy subjects, five male and two female. Their clinical history was readily obtained and checked during the course of the test and being competent to make clinical observations and reports themselves their personal experiences are of interest and are summarized.

These subjects reported for several days before being given the isopropanol, and their normal behavior, pulse rate, temperature, blood pressure, elimination and character of urine noted. Their eyes were also carefully examined and found perfect. At the end of the probationary period they were given doses of 20 to 30 c c isopropanol of 50 per cent strength, slightly sweetened and flavored to assist the unpalatable taste characteristic of an unflavored mixture of water and isopropanol.

In general the immediate effect of the administration of isopropanol was a lowering of the blood pressure, both systolic and diastolic. Sometimes, however, a rise was noted, and the variation from normal was not consistent. After about half an hour the diastolic returned to normal, and often showed an increase over that observed before administration. This was more noticeable with the male than with the female subjects.

The pulse pressure was lowered. The pulse rate varied, sometimes rising and sometimes falling, the effect often being different on the same subject on different days.

There was little effect on the respiration. In some cases, it rose a few points and again it went down.

All of the subjects reported that almost immediately after taking the dose, a sensation of warmth pervaded the system. They became dizzy to a greater or lesser degree, but this soon disappeared. One man and one woman experienced a tingling sensation, especially in the arms and legs, that lasted for some time, and two other male subjects reported an anesthetic or numbing reaction.

Drowsiness occurred in three cases on the first day of the test, but a tolerance seemed to be established and thereafter no condition of this character was experienced.

Five subjects experienced headache of varying intensity, one woman in particular awaking from a sound sleep with a severe pain.

In all cases, the symptoms were the most severe and lasting on the first day of the test, but thereafter the effects wore off in from one to three hours. In one instance aside from the sensation of warmth, slight dizziness, numbness and heaviness of eyelids which soon disappeared, there was no reaction experienced.

Each morning before reporting at the laboratory, the subjects were examined by the oculist and for two weeks after the dosages were stopped, observations of the eyes were continued. No untoward effects occurred, in fact, as was noted previously with every subject there was an apparent greater clarity of vision established.

Urine examinations made prior to ingestion of isopropanol gave negative tests for acetone. During the course of the experiment, acetone appeared, just as it had been observed in the case of the dogs. As soon as the subjects ceased taking isopropanol the acetone disappeared. The explanation of the presence of this body in the urine has already been discussed.

CONCLUSIONS

We have thus noted in brief the observations of the effects of isopropanol on the animal economy when this substance is taken into the system at a strength which can be ingested by the subject without apparent local discomfort.

The animal economy is capable of absorbing isopropanol in reasonable amounts without the accompaniment of toxic results.

That the ingestion of the substance produces a form of intoxication especially in the early period of the test is apparent, the violence and duration of the same depending on the species.

It is clear, however, that in most instances, barring perhaps cats, a tolerance is quickly established, and thereafter the outward appearance of the intoxication differs in no respect from that produced by ethyl alcohol.

ACTION ON MICROORGANISMS

Action of Isopropanol on Bacteria, Yeasts and Molds—The question may naturally arise as to what evidence we possess that isopropanol will act as a preservative against the action of yeasts, molds and bacteria. To answer this query, a series of tests was instituted, which demonstrated that isopropanol would inhibit the development of mold and render dormant the spores to the same degree at least that is done by ethyl alcohol. As to its comparative action against yeasts and bacteria, the following chart shows that it has an inhibitory value of greater intensity than ethyl alcohol.

In the test against bacteria, increasing quantities were mixed with standard extract broth and inoculated with *Bacillus typhosus*. After incubation subcultures were made whereby the disinfectant effect was determined. In the fermentation tests only the inhibitory values were recorded.

TABLE I
RESULTS, SHOWING THE COMPARATIVE ACTION OF ISOPROPANOL AND ETHYL ALCOHOL AGAINST BACTERIA AND YEASTS

BACTERIA					YEASTS		
Technic: Preparations in the following quantities were mixed with 10 c.c. of standard extract broth, inoculated with one standard (4 mm) loop of a vigorous 24 hour broth culture of <i>Bacillus typhosus</i> "Rawlings", cultured at 37.5 C for 48 hours and observed for inhibition subcultured on agar to determine disinfectant strength.					Technic: Preparations in the following percentages of the substances mixed with standard fermentation liquor were planted with bakers yeast and incubated 24 hours at 36 C.		
QUANTITY OF PREPARATION USED	INHIB CULT	SUB CULT	ETHYL ALCOHOL INHIB CULT	SUB CULT	PERCENTAGE MIXTURE	ISOPROPANOL	ETHYL ALCOHOL
c.c.							
1	+	+	+	+	7	+	+
2	+	+	+	+	8	+	+
3	+	+	+	+	9	+	+
4	±	+	+	+	10	+	+
5	±	+	±	+	11	+	+
6	-	-	±	+	12	+	+
7	-	-	-	+	13	+	+
8	-	-	-	-	14	-	+
9	-	-	-	-	15	-	+
10	-	-	-	-	16	-	+
11	-	-	-	-	17	-	-
12	-	-	-	-	18	-	-
13	-	-	-	-	19	-	-
14	-	-	-	-	20	-	-
15	-	-	-	-	21	-	-
					22	-	-
					23	-	-
					24	-	-
					25	-	-

Key: + indicates positive growth
- indicates no growth

DETAIL SUMMARY OF ACTION OF ISOPROPYL AND ETHYL ALCOHOLS ON ANIMALS

Rabbit Control—Observation started September 20, 1921, weight 2150 gm. Killed and autopsied November 25, 1921, weight 2225 gm. Behavior normal throughout period of observation. Gross pathology at autopsy: Some coccidiosis otherwise normal.

Rabbit No 1—Given 115 c.c. isopropyl alcohol in 5 c.c. doses. Observations begun Sept 28, weight, 2075 gm., to Oct 29, 1921, weight 2000 gm. Symptoms: Slight incoordination at first with little drowsiness, otherwise no appreciable effects. Tolerance established later. Gross pathology: Some evidence of mild chronic gastrointestinal catarrh, with chronic passive congestion otherwise apparently normal. Ophthalmologist reports both eyes probably a little blurred.

Chicken No 1a—Given 50 c.c. ethyl alcohol. Usual effect of this drug noted, larger dose (20 c.c.) produced drunkenness of less profound nature than isopropyl doses. About 6 hours duration. Still living and in apparently good health.

Rabbit No 2—Given 220 c.c. isopropyl alcohol in 10 c.c. doses. Observations begun Sept 28 (weight 1900), Oct 10, 1921 (weight 1630), to Nov 25, 1921 (weight 1900).

Oct 12, 1921, last dose Symptoms Considerable incoordination at first, gradually decreasing as apparent tolerance established, ate very little at first, later no particular effect on appetite Gross pathology Old, but mild chronic gastritis, otherwise no particular changes evidenced Ophthalmologist reports scar on left cornea and left disc decidedly blurred

Chicken No 2 a—Given 280 cc isopropyl alcohol in 10, 15 and 20 cc doses Observations begun Oct 20, 1921 Still living and in good condition Last dose 20 cc, Nov 30, 1921 Smaller doses produced well defined incoordination and symptoms of intoxication at first, 20 cc produced profound stupor lasting for 36 hours Considerable tolerance established toward last Lost weight and did not look healthy

Rabbit No 3—Given 90 cc of isopropyl alcohol in 15 cc doses At first showed rather rapid and pronounced incoordination and weakness of muscles, lasting 3 to 5 hours Tolerance established to some extent towards last Observations begun Sept 29th, 1921 (weight 2050), died from hemorrhagic lobar pneumonia 10 30 A M, October 6, 1921 (weight 1910)

Rabbit No 5—Given 170 cc isopropyl alcohol in 10 cc doses Observations begun Oct 4, 1921, (weight, 2050) to Oct 29, 1921, (weight, 1920) Weight, October 14, 1921, 2060 Symptoms similar to No 2 Ophthalmologist reports some cupping of disc, otherwise normal

Rabbit No 6—Given 135 cc isopropyl and 135 cc ethyl alcohol in 15 cc doses alternating every other day Observations begun Oct 4, 1921, (weight 2270) Killed and autopsied Oct 29, 1921 (weight 2000) Isopropyl produced quicker and more profound symptoms of intoxication, came on quicker and lasted longer Gross pathology showed some chronic gastritis and chronic passive congestion of liver and kidneys Ophthalmologist reported both cornea with parenchymatous changes and discs probably blurred

Rabbit No 7—Given 85 cc of ethyl alcohol in 5 cc doses Observations begun Oct 4, 1921 (weight, 2200), killed and autopsied Oct 29, 1921 (weight 2135) Symptoms Slight incoordination, some drowsiness and indisposition to eat at first, not noticeable toward last The ophthalmologist reported "Optic nerve yellowish, circulation good, cupping of disc marked" Gross pathology Some chronic gastritis and chronic passive congestion of liver and stomach

Rabbit No 8—Given 180 cc of ethyl alcohol in 10 cc doses Observations begun October 4, 1921 (weight 2005) Killed and autopsied Oct 29, 1921 (weight, 2050) Symptoms similar to above No 7, except more marked The ophthalmologist reported "Both cornea show marked interstitial changes" Gross pathology Stomach shows considerable chronic passive congestion, chronic gastritis and some mottling

Rabbit No 10—Given 210 cc isopropyl alcohol in 15 cc doses Observations begun Oct 7, 1921 (weight, 2200) Killed and autopsied Oct 29, 1921 (weight, 1870) Symptoms Pronounced incoordination, stupor well defined at first, fairly good tolerance established later The ophthalmologist reports "No changes noted" Gross pathology Stomach shows mild chronic gastritis with chronic passive congestion of liver, otherwise normal

Rabbit No 11—Given 105 cc ethyl alcohol and 105 cc isopropyl alcohol in alternate doses of 15 cc each Observations begun Oct 11, 1921 (weight, 2250) Killed and autopsied Oct 29, 1921 (weight, 2190) Symptoms Quite similar to No 6 Ophthalmologist reported "Optic disc whiter than normal i.e., less capillary circulation, cupping marked" Gross pathology Mild chronic passive congestion of stomach and liver

Black Cat No 28—Given 125 cc of isopropyl alcohol (50 per cent) in 20 cc and 15 cc doses, beginning November 18, 1921 (weight, 2056 gm) First dose 20 cc, Nov 18, 1921, 15 cc, Nov 22, 24, 25, 26, 28, 29, 30, 1921 Weight Nov 30, 1921, 1925 grams Animal very susceptible to the administration of the alcohol Exhibited marked incoordination from 5 to 8 minutes after administration, beginning first in the posterior extremities and gradually involving the whole musculature Animal became stuporous and 30 minutes later showed complete flaccid relaxation of muscles, respiration shallow and quite slow, ranging from 8 to 10 in the stuporous states During administration animal showed no apparent

tendency to establish tolerance to the alcohol. Was nauseated and showed no disposition to eat. Eyes examined Nov 29, 1921, no pathologic changes noted by the ophthalmologist. Animal killed and autopsied Nov 3, 1921. Thoracic and abdominal viscera showed considerable congestion, with subacute gastritis and enteritis, apparently from effects of the alcohol. Kidneys slightly swollen and congested. Liver showed some congestion with mild cloudy swelling. Lungs showed mild congestion, but no evidence of pneumonia or edema. Heart showed dilatation of right side. Brain shows mild congestion but otherwise apparently normal. Microscopic examination of kidneys, liver and brain confirm the gross examination. Cloudy swelling of kidneys, but no marked toxic effects. This animal, however, does not stand the administration of isopropyl alcohol as readily as dogs and rabbits.

White Dog No 29—Given 240 cc of isopropyl alcohol (50 per cent) in 30 c.c. doses. First dose given Nov 18, 1921, and Nov 21, 22, 25, 28, 29 and 30, 1921. Weight of animal not determined. (Could not be satisfactorily weighed on scales in use.) Animal showed considerable incoordination, beginning from 5 to 10 minutes after administration, first noted in the posterior extremities. This was accompanied by a moderate amount of muscular weakness, considerable slobbering was observed. Some indisposition to eat was noted at times, but toward the end of the administration of the drug had no particular effect. Animal lost some weight, apparent from gross examination. Tolerance well established toward the latter doses. Eyes were examined Nov 29, 1921, and no definite pathologic changes observed by the ophthalmologist. Dog showed some softening of the stool, but no evidence of passage of blood or other condition which might indicate gastroenteritis. After discontinuance of the drug animal made an uneventful recovery.

Gray Cat No 30—Given 20 cc (50 per cent) isopropyl alcohol, Nov 19 1921, 15 cc Nov 21, and 15 cc Nov 22, 1921. Animal showed marked incoordination after first administration, with considerable muscular weakness beginning first in the higher extremities and finally involving the entire musculature. In addition to loss of muscular control, seemed to lose sense of relationship, would butt into the wall of the cage. Finally developed stupor, with slowed respiration. The 15 cc doses did not produce quite so marked symptoms, but the usual symptoms were noted to a less extent. On Nov 22, animal died from aspiration pneumonia due to break in catheter. Mucous membranes of abdominal viscera showed some congestion but no necrosis or ulceration, also some congestion of the kidney and liver of a mild nature. Death due to aspiration pneumonia with edema (accidental).

Yellow Dog with Black Nose No 30—Tests begun Jan 19 1922, with isopropyl alcohol (50 per cent). Given 15 c.c. Jan 19, Jan 20, Jan 23, and 20 cc Jan 24, 15 cc Jan 25. Animal showed some incoordination after administration of the drug, with increased flow of saliva, some restlessness and irritation. Exhibited no particular indisposition to eat nor nausea. Stool became slightly softened. On Feb 2, examination of eyes showed no evidence of pathologic change, except possibly slight congestion of the retinae. Animal did not appear to lose any weight. Preparation was discontinued after January 25, and animal kept under observation. From January 25 to February 24 no untoward pathologic condition noted, appeared to be a healthy dog except for mange which developed over head and right shoulder. On February 24, administration of 50 per cent ethyl was begun. 15 c.c. doses being given on Feb 24, 25, 27 and March 1. On the administration of the alcohol, animal exhibited symptoms of incoordination similar to that caused by isopropyl but not so marked. Animal's eyes examined March 1 and found to be normal. Killed and autopsied March 1, 1922 with the following results. Exhibits mange over head shoulder and right side. Thoracic and abdominal viscera normal in position. Liver enlarged soft and friable, spleen normal. Stomach small walls somewhat thickened. Definite gastritis, quite similar to alcoholic gastritis seen in human adults. Intestines show some congestion of a chronic passive nature. Pancreas red and congested—no other abnormalities. Kidneys slightly swollen. Capsule strips with ease, cortex in fairly good condition. Heart shows dilatation of right ventricle, engorgement of right auricle. Lungs exhibit some chronic passive congestion. Brain exhibits a mild degree of congestion. No evidence of well defined pathology. Microscopic examination confirms the gross shows some interstitial hepatitis, similar to beginning alcoholic cirrhosis of the liver, and slight parenchymatous

nephritis of the kidneys Effects of alcohol on this animal are very similar to those seen in chronic ethyl alcohol poisoning in human adults

Large Yellow Dog, with White Paws, No 36—Tests begun January 19, 1922, with isopropyl alcohol (50 per cent); given 25 cc on Jan 19, 20, 22, 23, and 24 On February 25, began the administration of 50 per cent ethyl alcohol in 25 cc doses, being given Feb 25, 26, 27, 28, and March 1, 2, 3, 4, 6, 7, and 8 Isopropyl alcohol administration begun again on March 9, and given on the succeeding days of March 10, 11, and 14 Animal killed by ether and autopsied April 29, 1922 Animal showed symptoms very similar to that of No 30, with a tendency to establish some tolerance for the drug Isopropyl alcohol appeared to be slightly more toxic than ethyl alcohol No very serious pathologic manifestations were observed Eyes were examined on February 25 and March 7, but showed no evidence of optic atrophy or retinal destruction, although some congestion was present On autopsy the stomach showed chronic gastritis and chronic enteritis of long standing, quite similar to that seen in human adults who are alcohol addicts Liver showed some congestion and an increase in fibrous tissue changes Lungs and heart were grossly normal Kidney congested, capsule thickened, stripped with slight difficulty, some interstitial changes of a chronic nature observed Brain grossly normal except for a slight glazing of the pia mater Eyes grossly normal except for some congestion of the choroid and retinal coats Microscopic examination confirmed the gross findings, which indicate chronic changes produced by chronic alcoholism The changes observed in this animal were not in any way unusual from those seen in chronic cases of ethyl alcohol intoxication

ISOPROPYL ALCOHOL EXPERIMENTS ON HUMAN BEINGS

SUBJECT No 1 MALE

June 21 1922

Before Administration

Systolic blood pressure-----	125
Diastolic blood pressure-----	78
Pulse pressure -----	47
Pulse -----	92
Respiration -----	18

Dose at 12 52 P M of 20 cc 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

1 00 P M

Systolic blood pressure-----	115
Diastolic blood pressure-----	74
Pulse pressure -----	41
Pulse -----	88
Respiration -----	18

1 12 P M

Systolic blood pressure-----	114
Diastolic blood pressure-----	72
Pulse pressure -----	42
Pulse -----	72
Respiration -----	20

Symptoms as Reported by Subject

- 12 52 P M Burning sensation in throat
- 12 53 P M Sensation of warmth in stomach
- 1 15 P M Sensation of warmth in stomach still present
- 1 20 P M Eructation of gas

July 5, 1922

Before Administration

12 27 P M

Systolic blood pressure-----	120
12 20 P M Diastolic blood pressure-----	70
Pulse pressure -----	50
12 27 P M Pulse -----	76
Respiration -----	18

Dose at 12 30 P M of 30 cc 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms		12 47 P M	
	Systolic blood pressure	---	116
	Diastolic blood pressure	---	76
12 38 P M	Pulse pressure	---	46
	Pulse	---	68
12 47 P M	Respiration	---	16
		12 52 P M	
	Systolic blood pressure	---	108
	Diastolic blood pressure	---	74
12 52	Pulse pressure	---	34
	Pulse	---	66
	Respiration	---	16

Symptoms as Reported by Subject

12 31 P M	Burning sensation in throat
12 33 P M	Sensation of warmth in stomach
12 34 P M	Eruetation of gas
12 38 P M	Eruetation of gas
12 40 P M	Slight feeling of dizziness & lids of eyes feel heavy
12 47 P M	Eruetation of gas
12 53 P M	Eruetation of gas
1 30 P M	Eruetation of gas

July 6 1922

Before Administration		12 18 P M	
	Systolic blood pressure	-	116
	Diastolic blood pressure	---	78
	Pulse pressure	---	38
	Pulse	---	80
	Respiration	---	22

Dose at 12 20 P M of 30 cc 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms		12 35 P M	
	Systolic blood pressure	-	108
	Diastolic blood pressure	---	76
12 33 P M	Pulse pressure	---	32
	Pulse	---	76
12 50 P M	Respiration	---	20
		12 50 P M	
	Systolic blood pressure	---	108
	Diastolic blood pressure	---	78
12 48 P M	Pulse pressure	---	30
	Pulse	---	80
12 50 P M	Respiration	---	22

Symptoms as Reported by Subject

12 21 P M	Burning sensation in throat
12 22 P M	Sensation of warmth in stomach
12 23 P M	Eruetation of gas
12 27 P M	Eruetation of gas
12 30 P M	Sensation of warmth about face
12 37 P M	Sensation of heaviness of eyelids
12 38 P M	Eruetation of gas
12 50 P M	Increasing heaviness of eyes
	No other symptoms

July 7, 1922

Before Administration

12 55 P M

Systolic blood pressure.....	115
Diastolic blood pressure.....	80
Pulse pressure	35
Pulse	74
Respiration	18

Dose at 12 56 P M of 30 cc 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

1 11 P M

Systolic blood pressure	100
Diastolic blood pressure.....	76
Pulse pressure	24
Pulse	76
Respiration	18

1 26 P M

Systolic blood pressure.....	124
Diastolic blood pressure.....	76
Pulse pressure	48
Pulse	82
Respiration	20

1 37 P M

Systolic blood pressure.....	102
Diastolic blood pressure.....	74
Pulse pressure	28

Symptoms as Reported by Subject

- 12 56 P M Burning sensation in throat
 12 57 P M Eructation of gas
 1 04 P M Light feeling in head
 1 06 P M Anesthetic feeling
 1 14 P M Feeling of heaviness of eyelids
 No other symptoms

Urinalyses for Acetone

June 22, 1922, P M	Very good test
June 23, 1922, A M	Trace
June 24, 1922, 7 A M	Faint trace
July 5, 1922, P M	Good test
July 6, 1922, A M	Very good
July 6, 1922, P M	Strong, excellent test
July 7, 1922, A M	Fair
July 7, 1922, P M	Good
July 8, 1922, A M	Good
July 8, 1922, P M	Faint
July 9, 1922, A M	Faint
July 9, 1922, P M	Faint
July 10, 1922, A M	Faint
July 10, 1922, P M	Trace
July 11, 1922, A M	Faint trace

Eye Examinations

June 21, 1922

V O D = 20 15 cc = 20 15
 V O S = 20 100 cc = 20 15
 Fields normal for movement Fundi normal

June 26 1922

V O D = 20 15 with glass = 20 15

V O S = 20 70 with glass = 20 15

Field normal for form and motion Fundi normal

No evidence of any change since last examination

July 8 1922

Eyes examined Vision same No change in either eye

SUBJECT No 2 MALE

June 21 1922

Before Administration

Systolic blood pressure	115
Diastolic blood pressure	55
Pulse pressure	60
Pulse	84
Respiration	18

Dose at 12 40 P M of 20 c c 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

12 55 P M

Systolic blood pressure	106
Diastolic blood pressure	48
Pulse pressure	58
Pulse	68
Respiration	16

1 08 P M

Systolic blood pressure	104
Diastolic blood pressure	48
Pulse pressure	50
Pulse	72
Respiration	10

Symptoms as Reported by Subject

12 40 P M Burning sensation in mouth and stomach
 12 45 P M Dull ache in stomach slight hightheaded sensation and very slight
 dizziness beginning of feeling of warmth to body
 12 52 P M Slight bitemporal headache
 1 00 P M Headache very slight
 1 05 P M Dizziness almost gone
 1 11 P M Headache disappeared
 1 15 P M Slight feeling of mental and physical dullness are only symptoms
 noticeable

July 5 1922

Before Administration

12 20 P M

Systolic blood pressure	117
12 15 P M Diastolic blood pressure	55
Pulse pressure	62
12 20 P M Respiration	20
Pulse	70

Dose at 12 27 P M of 30 c c 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

12 40 P M

Systolic blood pressure	96
12 33 P M Diastolic blood pressure	54
Pulse pressure	42
Pulse	70
12 40 P M Respiration	14

12 56 P M

	Systolic blood pressure.....	98
12 48 P M	Diastolic blood pressure.....	56
	Pulse pressure	42
	Pulse	68
12 56 P M	Respiration	16

Symptoms as Reported by Subject

12 27 P M	Burning sensation from mouth to stomach	
12 33 P M	Slight eructation of gas	
12 37 P M	Feeling of slight dizziness	
12 42 P M	Sensation of light headedness, very slight headache	Eyes feel heavy with desire to close them
12 47 P M	Began to feel sleepy	
12 57 P M	More dizzy and much more sleepy, sleepiness progressing until about 3 P M	Sleepy
	No other symptoms after 3 P M	

July 6, 1922

Before Administration

	Systolic blood pressure.....	98
	Diastolic blood pressure.....	58
12 24 P M	Pulse pressure	40
	Pulse	76
	Respiration	16

Dose at 12 24 P M of 30 c c 50 per cent isopropyl alcohol solution by mouth

Objective Symptoms

12 39 P M

	Systolic blood pressure.....	94
	Diastolic blood pressure.....	48
12 55 P M	Pulse pressure	44
	Pulse	78
	Respiration	20

12 55 P M

	Systolic blood pressure.....	96
	Diastolic blood pressure.....	52
12 55 P M	Pulse pressure	44
	Pulse	80
	Respiration	20

Symptoms as Reported by Subject

12 26 P M	Burning sensation in throat	
12 29 P M	Feeling of warmth in stomach	
12 30 P M	Feeling of haziness and dizziness	
12 35 P M	Head feels very heavy	Sensation of warmth all over body
12 40 P M	Increasing dizziness, head feels large and full	
12 46 P M	Cannot walk a straight line	
12 48 P M	No headache at all, feel sleepy and head feels larger and heavier	
1 30 P M	Very slight drowsiness remaining	
2 00 P M	Cessation of all symptoms	

July 7, 1922

Before Administration

12 52 P M

	Systolic blood pressure.....	100
	Diastolic blood pressure.....	50
12 52 P M	Pulse pressure	50
	Pulse	74
	Respiration	18

Dose at 12 54 P M of 30 cc 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

1 09 P M

	Systolic blood pressure.....	100
	Diastolic blood pressure.....	44
1 09 P M	Pulse pressure.....	56
	Pulso.....	66
	Respiration.....	18

1 24 P M

	Systolic blood pressure.....	96
	Diastolic blood pressure.....	42
1 24 P M	Pulse pressure.....	54
	Pulso.....	64
	Respiration.....	16

1 33 P M

	Systolic blood pressure.....	94
1 33 P M	Diastolic blood pressure.....	48
	Pulse pressure.....	46

Symptoms as Reported by Subject

- 12 55 P M Burning sensation in throat and warmth in stomach Eructations of gas
- 1 15 P M Do not feel any of the symptoms of dizziness lightheadedness as in previous experiments
- 1 25 P M Very slight feeling of lightheadness in head and fullness in eyes
- 1 45 P M Cessation of all symptom No further symptoms

Urinalyses for Acetone

June 22, 1922, 6 P M Very good test

June 23, 1922, 7 A M Very good test

June 24, 1922 7 A M Very good test

July 5, 1922, P M Trace

July 6, 1922, A M Good test

July 6, 1922 P M Good

July 7, 1922, A M Very strong

July 7, 1922 P M Very good

July 8, 1922, A M Good

July 8, 1922, P M Faint

July 9, 1922 A M Faint

July 9, 1922 P M Faint

July 10, 1922, A M Faint

July 10, 1922 P M None

July 11, 1922, A M None

Eye Examinations

June 21 1922

V O D = 20-30 -1 = 20-30 with glass

V O S = 20-30 -1 = 20-30 with glass

Fields normal for movement Fundi normal

June 26 1922

V O D = 20-20 -3 = 20-20 with glass

V O S = 20-20 -1 = 20-20 with glass

Fields normal for movement and motion Fundi normal

No evidence of any change since last examination

July 8 1922

Eyes examined Vision better No change in eye

SUBJECT NO 3 MALE

July 5, 1922

Before Administration		12 31 P M	
	Systolic blood pressure.....	110	
12 25 P M	Diastolic blood pressure.....	62	
	Pulse pressure	48	
	Pulse	62	
12 31 P M	Respiration	20	

Dose at 12 35 P M. of 30 c c 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms		12 40 P M	
	Systolic blood pressure.....	100	
	Diastolic blood pressure.....	62	
	Pulse pressure	38	
	Pulse	66	
	Respiration	16	

		1 05 P M	
	Systolic blood pressure.....	102	
12 57 P M	Diastolic blood pressure.....	64	
	Pulse pressure	38	
	Pulse	60	
1 05 P M	Respiration	14	

Symptoms as Reported by Subject

12 35 P M	Burning taste, burning sensation in nostrils
12 36 P M	Sense of warmth in esophagus and stomach
12 39 P M	Eruetation of gas
12 45 P M	Slight sensation of lightness of head when standing
12 52 P M	Slight dizziness
12 56 P M	Slight drowsiness
1 30 P M	Drowsiness and dizziness disappeared
3 15 P M	Slight headache (frontal) especially about eyes, eruclatations of gas
7 00 P M	Headache quite severe

July 6, 1922

Before Administration		12 15 P M	
	Systolic blood pressure.....	108	
12 13 P M	Diastolic blood pressure.....	62	
	Pulse pressure	46	
	Pulse	60	
12 15 P M	Respiration	20	

Dose at 12 18 P M of 30 c c 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms		12 35 P M	
	Systolic blood pressure.....	92	
12 29 P M	Diastolic blood pressure.....	60	
	Pulse pressure	32	
	Pulse	62	
12 35 P M	Respiration	16	

		12 47 P M	
	Systolic blood pressure.....	88	
12 44 P M	Diastolic blood pressure.....	66	
	Pulse pressure	22	
12 47 P M	Pulse	60	
	Respiration	20	

1 00 P M

Systolic blood pressure	98
Diastolic blood pressure	68
Pulse pressure	30

Symptoms Reported by Subject

- 12 19 P M. Burning sensation in esophagus and stomach, sense of warmth in stomach
- 12 32 P M Slight dizziness
- 12 35 P M Heavy feeling about eyes
- 12 44 P M Sensation of intoxication
- 1 00 P M Slight headache
- 2 10 P M Disappearance of all symptoms

July 7 1922

Before Administration

12 44 P M

Systolic blood pressure	105
Diastolic blood pressure	65
Pulse pressure	40
Pulse	66
Respiration	18

Dose at 12 49 P M of 30 cc 50 per cent solution isopropyl alcohol by mouth.

Objective Symptoms

1 04 P M

Systolic blood pressure	98
Diastolic blood pressure	70
Pulse pressure	28
Pulse	60
Respiration	16

1 19 P M

Systolic blood pressure	100
Diastolic blood pressure	66
Pulse pressure	34
Pulse	60
Respiration	16

Symptoms Reported by Subject

- 12 50 P M Burning sensation in esophagus and stomach
- 12 58 P M Sensation of light feeling in head eructation of gas
- 1 11 P M Slight dizziness and heaviness of eyelids
- 1 30 P M Cessation of symptoms

Urinalyses for Acetone

July 5, 1922, P M	Very good
July 6, 1922, A M	Good test
July 6, 1922 P M	Good
July 7, 1922, A M	Very good
July 7, 1922 P M	Good
July 8, 1922 A M	Good
July 8, 1922 P M	Marked trace
July 9 1922, A M.	Faint
July 9, 1922 P M	Faint
July 10, 1922 A M	Faint
July 10 1922 P M	None
July 11 1922 A M	Very faint

Eye Examinations

July 8 1922

- V O D 20-20 with glass 20-30
- V O S 20-20 with glass 20-30
- No pathologic change noted in either eye

SUBJECT NO 4 MALE

April 22, 1922

Before Administration

Pulse -----	72
Respiration -----	26

Dose at 12 20 P M of 10 c c isopropyl alcohol by mouth

Objective Symptoms

12 37 P M

Pulse -----	80
Respiration -----	26

12 45 P M

Pulse -----	78
Respiration -----	24

Symptoms as Reported by Patient

- 12 37 P M Slight sensation of flush Eyes feel heavy
 Very slight dizziness on standing
 Sensation of warmth general
 Mild sensation of dryness of mouth
- 1 30 P M Slight nausea in stomach
- 2 00 P M Dull headache (slight)
- 2 40 P M Voided cloudy urine
- 3 15 P M Headache gone, food taken
- 5 25 P M Voided cloudy urine
- 7 00 P M Voided slightly clouded urine
- 10 00 P M Voided clear urine

April 23, 1922

- 1 00 A M Voided clear urine
- 7 30 A M Voided clear urine
- 9 30 A M Voided clear urine
- 12 00 NOON Voided clear urine

July 5, 1922

Before Administration

12 28 P M

Systolic blood pressure -----	102
Diastolic blood pressure -----	70
Pulse pressure -----	32
Pulse -----	72
Respiration -----	22

Dose at 12 38 P M of 30 c c 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

12 45 P M

Systolic blood pressure -----	88
Diastolic blood pressure -----	62
Pulse pressure -----	26
Pulse -----	80
Respiration -----	24

1 00 P M

Systolic blood pressure -----	90
Diastolic blood pressure -----	65
Pulse pressure -----	25
Pulse -----	80
Respiration -----	24

Symptoms as Reported by Subject

- 12 40 P M Slight burning sensation in stomach.
- 12 42 P M Eructation of gas

12 43 P M Feeling of dizziness
 12 44 P M Ringing sensation in ears
 12 45 P M Anesthetic feeling to skin
 12 46 P M Feeling of dizziness subsiding, ringing sensation in ears gone
 12 54 P M Feeling of drowsiness, gas in stomach
 12 57 P M Normal feeling returning
 1 20 P M Headache and increased drowsiness
 3 30 P M Severe headache and drowsiness

Slept from 7 15 P M until 5 05 A.M., awoke with headache voided clear urine
 Summary of night Was very dopy all evening went to bed with a dull headache Slept
 very well awoke with the same headache, dry throat, no loss of appetite
 9 00 P M July 6, 1922 Headache gone

July 6 1922

Before Administration

12 10 P M

Systolic blood pressure	94
Diastolic blood pressure	60
Pulse pressure	28
Pulse	80
Respiration	24

Dose at 12 13 P M of 30 c.c. 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

12 25 P M

Systolic blood pressure	96
Diastolic blood pressure	72
Pulse pressure	24
Pulse	80
Respiration	22

12 43 P M

Systolic blood pressure	95
Diastolic blood pressure	70
Pulse pressure	25
Pulse	72
Respiration	24

Symptoms as Reported by Subject

12 15 P M. Slight burning sensation in stomach
 12 16 P M Eructation of gas
 12 22 P M Slight sensation of dizziness
 12 28 P M Increased dizziness—numbness
 12 31 P M Warm sensation over body
 12 32 P M Dizziness lessening
 12 35 P M Slight nausea in stomach
 12 37 P M Feeling of drowsiness
 12 38 P M Feeling of drowsiness increasing
 12 47 P M Normal feeling returning
 1 30 P M All effect wearing off
 2 30 P M No effect Feel normal

July 7 1922

Before Administration

12 54 P M

Systolic blood pressure	98
Diastolic blood pressure	70
Pulse pressure	28
Pulse	80
Respiration	20

Dose at 12 50 P M of 30 c.c. 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

1 14 P M

Systolic blood pressure-----	100
Diastolic blood pressure-----	72
Pulse pressure -----	28
Pulse -----	82
Respiration -----	20

1 29 P M

Systolic blood pressure-----	96
Diastolic blood pressure-----	70
Pulse pressure -----	26
Pulse -----	80
Respiration -----	22

1 40 P M

Pulse -----	78
Respiration -----	18

Symptoms as Reported by Subject

12 59 P M	Tastes very hot, leaving burning sensation in throat
1 02 P M	Burning sensation in stomach
1 03 P M	Slight dizziness
1 04 P M	Increased dizziness with ringing sensation in ears
1 05 P M	Dizziness lessening, with perspiration
1 06 P M	Increased ringing sensation in ears
1 07 P M	Anesthetic feeling to body
1 08 P M	Increased feeling of numbness
1 11 P M	Eruetation of gas
1 17 P M	Increased sensation of dizziness
1 20 P M	Slight inclination to giddiness
1 25 P M	Normal feeling returning
1 30 P M	Normal feeling after lunch at this time
1 30 P M to midnight	Excessive odor of whiskey to breath

Urinanalysis for Acetone

July 5, 1922, P M	Very good test
July 6, 1922, A M	Good test
July 6, 1922, P M	None submitted
July 7, 1922, A M	Good test
July 7, 1922, P M	Fair
July 8, 1922, A M	Fair
July 8, 1922, P M	Marked trace
July 9, 1922, A M	Faint
July 9, 1922, P M	Faint
July 10, 1922	None submitted

Eye Examinations

April 22, 1922

Eyes examined and found normal Attention called to grave possibilities in experimenting with humans with alcohols—patient did not seem to appreciate possibilities according to oculist

July 6, 1922

V O D 20-30 with glass 20-30	
V O S 20-25 with glass 20-30	
Fields normal for movement	Fundi normal

July 8, 1922

Eyes examined No changes of pathologic nature noted

SUBJECT No 5 MALE

June 22 1922

Before Administration

Systolic blood pressure	108
Diastolic blood pressure	80
Pulse pressure	28
Pulse	68
Respiration	24

Dose at 12 37 P M of 20 c.c. 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

12 40 P M

Systolic blood pressure	106
Diastolic blood pressure	80
Pulse pressure	26
Pulse	76
Respiration	28

1 04 P M

Systolic blood pressure	106
Diastolic blood pressure	80
Pulse pressure	26
Pulse	72
Respiration	16

Symptoms as Reported by Subject

12 39 P M Sensation of warmth in stomach sensation of dizziness and slight loss of tactile sensation in fingers

12 50 P M Warmth in stomach still persist Still somewhat dizzy Loss of sensation in fingers still present

1 05 P M Slight headache

1 15 P M Headache somewhat increased but sensation of numbness leaving fingers. Feeling somewhat 'dopy'

1 30 P M Headache decreasing and feeling practically normal

Urinalyses for Acetone

June 22, 1922, 7 50 P M Very good test

June 23, 1922 9 20 A M Very good test

June 23, 1922 8 00 P M Trace

June 24, 1922 9 00 A M None

Eye Examinations

June 22 1922

Fields normal for movement Fundi normal

July 8 1922

All examinations normal

SUBJECT No 6 FEMALE

July 14 1922

Before Administration

1 35 P M

Systolic blood pressure	132
Diastolic blood pressure	80
Pulse pressure	52
Pulse	80
Respiration	18

Dose at 1 50 of 30 c.c. 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

2 18 P M

Systolic blood pressure	124
Diastolic blood pressure	78
Pulse pressure	46
Pulse	76
Respiration	18

2 23 P M

Systolic blood pressure-----	108
Diastolic blood pressure-----	72
Pulse pressure -----	36
Pulse -----	80
Respiration -----	20

Symptoms as Reported by Subject

- 1 40 P M Disagreeable taste, afterwards resembling whiskey taste
- 2 15 P M Sensation of warmth, slight tingling sensation in hands and feet which spreads over body and becomes slightly accelerated
- 2 15 P M Eructation of gas with smoky taste, disappearance of sensation of wry neck
- 3 15 P M Sensation of pressure on top of head, feeling of relaxation with cold, clammy perspiration over body
- 4 00 P M Headache, not severe, also slight nausea
- 5 30 P M Sensation of hunger and thirst, dinner
- 7 00 P M Normal feeling with exception of sensation of slight pressure on top of head, metallic taste in mouth all afternoon, increase of saliva, kidneys very active, amount of urine increased

Urinalysis for Acetone

July 14, 1922, A M	None
July 14, 1922, P M	Good test
July 15, 1922, A M	Very good
July 15, 1922, P M	Faint
July 16, 1922, A M	None
July 16, 1922, P M	Very faint
July 17, 1922, A M	None

SUBJECT No 7 FEMALE

July 14, 1922

Before Administration

1 48 P M

Systolic blood pressure-----	120
Diastolic blood pressure-----	84
Pulse pressure -----	36
Pulse -----	72
Respiration -----	24

Dose at 1 55 P M of 30 c c 50 per cent solution isopropyl alcohol by mouth

Objective Symptoms

2 12 P M

Systolic blood pressure-----	118
Diastolic blood pressure-----	76
Pulse pressure -----	42
Pulse -----	80
Respiration -----	24

2 27 P M

Systolic blood pressure-----	112
Diastolic blood pressure-----	74
Pulse pressure -----	38
Pulse -----	76
Respiration -----	24

Symptoms as Reported by Subject

Dose at 1 55 P M No symptoms from medicine for ten minutes except slight stinging sensations to mouth After ten minutes hands became cold and clammy and perspiration broke out over chest and head, particularly upper lip Thirty minutes after a sense of lightness (rather slight) was experienced in the head and a rather carefree feeling, also a slight

tingling sensation in legs and arms which remained for about two hours. No further symptoms until 4:30 A M, July 15, 1922, when she awoke with a very severe headache which could only be relieved with 10 grains of aspirin. No other symptoms.

Urinalyses for Acetone

July 14, 1922, A M	None
July 14, 1922, P M	Good test
July 15, 1922, A M	Good
July 15, 1922, P M	Not collected
July 16, 1922, A M	Not collected
July 16, 1922, P M	Not collected
July 17, 1922, A M	None

STUDIES ON NEPHRITIS*

I. PHYSIOLOGIC AND ANATOMIC CHANGES FOLLOWING TEMPORARY ISCHEMIA OF THE KIDNEYS

By E. T. McENERY, M.S., M.D., JACOB MEYER, M.D., AND A. C. IVY, M.D.
CHICAGO, ILL.

THIS work was undertaken as preliminary to the study of gastric secretory changes which occur in nephritis. As we desired to produce a nephritis without using toxins or chemicals which per se might alter gastric secretion, we studied the effects of temporary occlusion of the renal blood vessels on the kidney, hoping thereby to produce a condition simulating nephritis.

Rowntree, Fitz and Geraghty¹ reviewed the literature up to 1913 and concluded that three facts had been established, namely, that following the complete or partial obstruction of the venous return from the kidney there results (a) albuminuria (b) hematuria if the lumen of the vein be greatly narrowed, and (c) the appearance of epithelial cells singly, in groups, or as casts in the urine.

They found, on occluding the vein sufficiently to cause a congestion of considerable degree, that albuminuria occurred almost constantly, that casts and red cells were usually present and that the phthalein and sodium chloride elimination was decreased as was sometimes the amount of urine. They also found that sometimes more urine (low solids) was excreted from the congested kidney than from the normal kidney. In some cases on histologic examination they found in the chronically congested kidney small abscesses and in one case, an increase in connective tissue but on the whole they concluded that "chronic passive congestion of varying intensity is produced without an accompanying chronic nephritis." Guthrie² found that complete ischemia of the cat's kidney for ten minutes was much less harmful than perfusion of the kidney with normal saline or Ringer's solution. In one instance he observed almost complete degeneration of a kidney that had been perfused for about nine minutes. Eisendrath and Strauss³ observed that temporary

compression of the renal vessels of rabbits for thirty minutes or less caused very slight changes, but if continued for forty-five minutes or more, definite parenchymatous degeneration and interstitial infiltration resulted. Many observers—Litten,⁵ Thorel,¹⁰ Foa,² MacNider,⁶ Bradford¹—have studied and described the anatomic picture resulting from permanently ligating and clamping for long periods (one and one-half to twenty-four hours), all the kidney vessels or various branches of them. More recently Marshall and Crane⁷ have reported that anemia of the kidney of short duration does not cause a prolonged anuria, and that anemia of one to three minutes has no effect on excretion of urine except for the appearance of protein in the urine. They found, however, that an anemia of twenty to twenty-five minutes caused decrease in the elimination of urea, phosphate, sulphate, creatinine and ammonia. Stoll and Carlson⁸ performed a great number of experiments and found that anemia of the kidney for periods of from one to twenty minutes, caused anuria for varying periods following the release of the clamped vessels, and that the urine when it did appear, was distinctly dilute in character. They further observed a prolonged spasm of the renal vessels on releasing the renal artery after occlusion.

No observations have been made on the blood urea following ischemia of the kidneys, and the urine changes have not been followed for a long period of time following temporary occlusion (thirty to forty-five minutes).

METHODS

Female dogs were operated on (perineorrhaphy) in such a manner as to expose the urethral orifice. Such animals could be easily and aseptically catheterized. The dogs were put on a standard maintenance diet consisting of meat, bread and milk. (Control observations were made for from five to ten days prior to the operation at which the renal vessels were occluded.)

Blood urea was determined by Marshall's urease method. The specific gravity, acidity, chlorides, albumin, urea, and total nitrogen of the urine were determined. The elimination of phenolsulphonephthalein by the kidneys was studied, 250 cc of water being given by stomach tube prior to injecting the dye.

The blood vessels were occluded with a "bull-dog" clamp. The vessels between the clamp and kidney were palpated for pulsation after clamping and before removal of the clamp, in order to make certain that the clamp was and had been functioning. Occlusion was maintained for periods varying from one-half an hour to one hour, the abdomen being temporarily closed to prevent exposure of the intestines.

RESULTS

We have made observations on twenty dogs following occlusion of the vessels of the kidney for varying periods. In eight dogs, the blood urea was determined, the urine analyzed, and the phenolsulphonephthalein elimination followed, frequently. In twelve dogs the vessels of both kidneys were clamped for forty-five minutes, but only the objective symptoms, dye elimination, and pathologic anatomy were observed.

We will give very brief protocols of the eight dogs that we studied in more detail. The chlorides are expressed in grams per 100 cc, the albumin, in grams per liter (Esbach), the phenolsulphonephthalein elimination, in per cent eliminated in two hours, and the blood urea, in grams per 100 cc.

Protocol Dog 2—Vessels of both kidneys occluded for thirty minutes

Nov 21 to Dec 8 Preliminary control period on a maintenance diet. Quantity of urine, 100 to 190 cc sp gr, 1040 to 1080 chlorides, 12 to 33, no albumin, no sugar, phenolsulphonephthalein elimination 90 to 95, blood urea 0.028 to 0.030

Dec 8 Occluded blood vessels of both kidneys for one half an hour

Dec 9 Eighty two cc urine by catheter, sp gr 1050 chlorides 10 albumin, 0.4 no sugar, phenolsulphonephthalein elimination 87 blood urea, 0.077, anorexia, and some vomiting

Dec 12 Urine, 97 cc sp gr 1050 chlorides 0.85 albumin 0.4 no sugar, phenol sulphonephthalein elimination 80

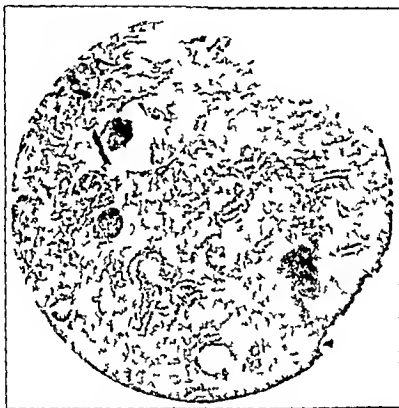


Fig 1—Microphotograph from the right kidney of Dog 2 showing two dilated glomerular spaces filled with hyaline material and red blood corpuscles with absence of the glomerular tuft.

Dec 15 Urine, 60 cc albumin 2.6 blood urea 0.067 Dog is losing weight Does not eat much and vomits occasionally

Dec 15 to Jan 3 Average amount of urine 10 cc

Jan 3 Thirteen cc of urine by catheter sp gr 1040 chlorides 0.67 albumin, 2.0 phenolsulphonephthalein elimination 60 blood urea 0.130 very depressed and weak coma but no convulsions

Jan 4 Death Autopsy No gross pathology present except that observed in the kidneys. Kidney vessels normal. Left kidney was less than half the normal size. Right kidney was normal in size and there were no gross changes on section. Histology Left kidney shows areas of hyaline degeneration congestion of and old hemorrhages about the tubules (clumps of hemotoidin) multiple small abscesses chiefly in the medulla. Many blood vessels contain thrombi some of which are being organized. Right kidney shows cellular detritus and casts present in a few of the tubules. Tubular epithelium in places shows degeneration and desquamation. A few of the glomeruli show degenerative changes but they are not as marked as those in the tubules. There is some increase in medullary connective tissue.

Protocol, Dog 3—Vessels of both kidneys occluded for forty five minutes, followed six months later by removal of one kidney and trauma of the other

Dec 24 to 27 Preliminary control period on a maintenance diet Quantity of urine, 150 to 175 cc, sp gr, 1040, chlorides, 135 to 166, no albumin, no sugar, phenolsulphonephthalein elimination, 90 to 95, blood urea, 0.040 to 0.043 Weight 11.8 kg

Dec 27 Occluded vessels of both kidneys forty five min

Dec 28 No spontaneous urine Forty five cc urine by catheter, sp gr, 1050, chlorides, 0.83, albumin, 1.6, no sugar, phenolsulphonephthalein elimination, 40, blood urea, 0.043, anorexia, but no vomiting

Dec 31 Quantity, 275 cc, sp gr, 1030, chlorides, 0.99, albumin, 0.5, phenolsulphonephthalein elimination, 80, blood urea, 0.030, appetite normal

Dec 31 to March 12 Observed daily Normal

March 12 Quantity, 300 cc, sp gr, 1035, chlorides, 0.65, albumin, 0.5, phenolsulphonephthalein elimination, 70

May 20 Quantity, 425 cc, chlorides, 0.45, albumin, negative, phenolsulphonephthalein elimination, 68, blood urea, 0.04 Animal in splendid condition

June 25 Same as above, except for phenolsulphonephthalein elimination, which is now 64

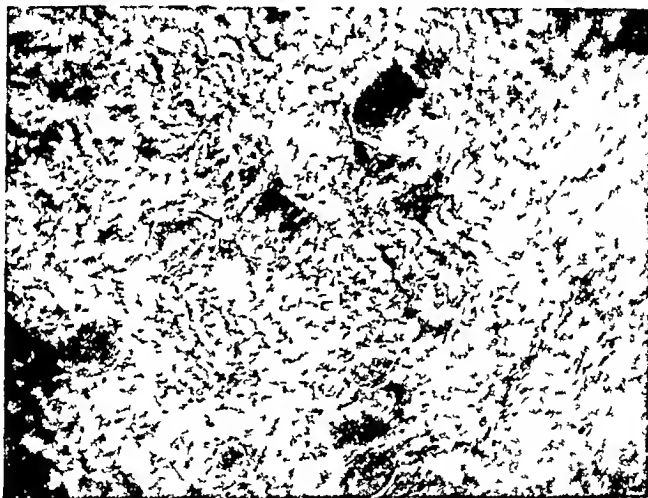


Fig 2—Microphotograph from the left contracted kidney of Dog 2 showing hemorrhage into the glomerular tuft and about the tubules and hyaline degeneration of the tubular epithelium Other portions of the same section show connective tissue and lymphocytic infiltration about the glomeruli and hyaline material in the tubules

July 17 Quantity, 325 cc, sp gr, 1040, chlorides, 0.88, albumin, negative, phenolsulphonephthalein elimination, 64, blood urea, 0.035, physical condition excellent, weight, 16.2 kg

July 22 Operation. Left kidney, which was less than one half the normal size, was removed Kidney vessels normal The right kidney was bound to the liver and intestine by adhesions, which were severed

July 23 Thirty five cc of urine by catheter, sp gr, 1055, chlorides, 0.76, albumin, 0.5, phenolsulphonephthalein elimination, 42, blood urea, 0.030

July 24 Urine, 445 cc, sp gr, 1040, chlorides, 0.71, albumin, 0.5, phenolsulphonephthalein elimination, 57, blood urea, 0.076 Dog does not eat and has an abscess of the mammary gland

July 26 Urine, 325 cc, sp gr, 1030, chlorides, 0.21, albumin, 1.0, phenolsulphonephthalein elimination, 34, blood urea, 0.141, dog vomits and does not eat

July 28 Death Autopsy Peritonitis secondary to abscess of mammary gland, which infected the abdominal incision The remaining kidney was of normal size, soft in consistency, congested, and the pelvis contained pus Histology The left kidney which was re-

moved at the operation shows an increase in connective tissue in areas chiefly in the medulla. There is an increase in the connective tissue about some of the glomeruli

Protocol Dog 4—Vessels of both kidneys occluded for one hour

Jan 8 to 21 Preliminary control period on diet Quantity of urine, 300 to 350 sp gr, 1020 to 1035, chlorides, 0.60 to 1.07, blood urea 0.032 to 0.050, no albumin, sugar negative phenolsulphonephthalein elimination 70 to 75

Jan 21 Occluded blood vessels of both kidneys for one hour Body weight, 8.5 kg

Jan 22 Anuria for twenty four hours Drinks water but vomits

Jan 23 Some vomiting and anorexia Quantity of urine, 190 c.c. sp gr 1015, chlorides, 0.31, blood urea, 0.121 albumin 0.5 no sugar phenolsulphonephthalein elimination, 10, urino sediment, many kidney cells few polymorph no casts or red blood cells, mucous shreds

Jan 25 to Feb 3 Quantity of urine 350 to 550 c.c. other findings about the same as above except for phenolsulphonephthalein elimination which is 23 Dog is losing weight and has a very poor appetite



Fig 3—Microphotograph from the left kidney of Dog 3 showing an increase in the connective tissue about the glomerulus.

Feb 9 Urine quantity, 300 c.c. sp gr, 10.0, chlorides 10, blood urea, 0.070 albumin, 0.05 phenolsulphonephthalein elimination 66 Dog has gained weight and has a normal appetite

Feb 9 to April 9 Observations continued as above weight 10.8 kg

April 9 to July 8 Quantity of urine 420 to 650 c.c. sp gr 1020 to 1025, chlorides, 0.7 to 0.9, blood urea, 0.040, albumin negative phenolsulphonephthalein elimination 67

July 11 Dog accidentally killed Both kidneys were white and contracted but only slightly less than normal in size The cortex was pitted with areas of pale dense tough tissue No tissue was saved for histologic examination Renal arteries and veins normal

Protocol Dog 5—Vessels of both kidneys occluded for one hour

Feb 8 to Feb 13 Preliminary control period on diet Quantity of urine, 430 to 450 c.c., sp gr, 1010 to 1015 chlorides, 1.0 to 1.25 blood urea, 0.074 to 0.041, no albumin, phenolsulphonephthalein elimination, 85 to 88 weight 11.2 kg

Feb 14 Occluded blood vessels for one hour

Feb 15 Anuria and some vomiting

Feb 16 Quantity, 100 c.c. sp gr, 1008 chlorides 0.23 blood urea, 0.123 albumin, 0.5 no sugar phenolsulphonephthalein elimination, 34 Dog does not eat.

Feb 18 to 20 Quantity 250 to 375 c.c. sp gr, 1011 to 1012 chlorides 0.34 to 0.60 blood urea 0.055 to 0.066 albumin, 0.5, phenolsulphonephthalein elimination 69 Smear of

urine sediment, renal cells, cellular casts, red blood cells and a few polymorphs Dog does not eat

Feb 23 Quantity, 140 cc, sp gr, 1018, chlorides, 0.35, blood urea, 0.093, albumin, 1.25 Smear of urine sediment, renal cells, but no blood Anorexia and some vomiting

Feb 25 Same as above, except for albumin, which is 1.5 Weight of dog, 9.4 kg Vomiting daily

Feb 27 Vomiting Marked weakness and depression, followed by coma

Feb 28 Dog died Autopsy The left kidney is of normal size On section it presents evidence of congestion and several small areas that appear to be necrotic. The right kidney is about half normal in size and has a pitted and speckled appearance On section, many areas resembling small abscesses are seen Approximately one fourth of the kidney appears normal grossly Renal arteries and veins normal The pelvis of each kidney contains a small quantity of turbid fluid, but there is no evidence of inflammation Urinary bladder normal Ten cc of bloody fluid is in the pericardial space The valve cusps are hyperemic. Five cc of clear fluid is present in each pleural cavity The lungs markedly are everywhere edematous and congested Ecchymotic hemorrhages are present in the mucous membrane of the stomach and colon The brain is congested and more moist than normal

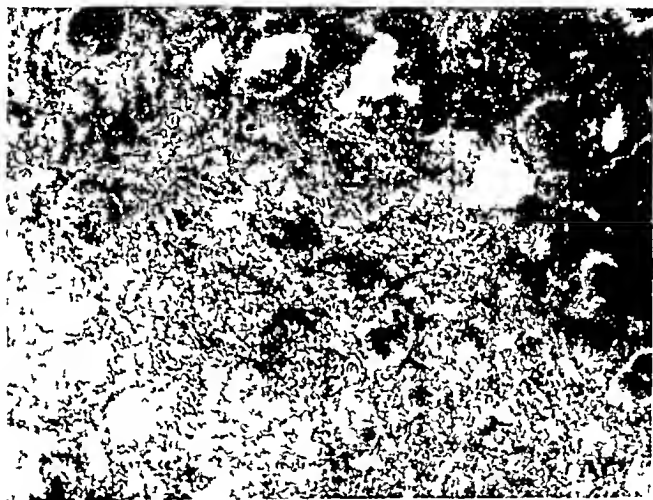


Fig 4—Microphotograph from the right kidney of Dog 5 showing marked degeneration of the tubular epithelium and hyaline material in the lumen of the tubules

Histology of kidneys Right kidney Blood vessels of pyramids congested Marked degenerative changes are present in some tubules Hyaline degeneration is present in convoluted portion of other tubules There are areas of tubular necrosis that are beginning to become calcified Hyaline and cellular casts are present in some tubules Left kidney There are a few tubules that show degenerative changes, and a few areas of tubular necrosis showing evidence of calcification Some of the glomeruli are hemorrhagic and white blood cells can be seen in the proximal tubules The walls of the blood vessels are thickened in both kidneys and a few thrombi can be seen in the small arteries

Protocol, Dog 6—Blood vessels of both kidneys occluded one hour

Feb 25 to March 3 Preliminary control period on diet Quantity of urine, 710 to 825 cc, sp gr, 1020 to 1025, chlorides, 0.50 to 1.5, blood urea, 0.034 to 0.037, no albumin, no sugar, phenolsulphonephthalein elimination, 92 to 94

March 3 Occluded vessels of both kidneys for one hour

March 4 Does not eat, but vomits occasionally

March 5 Quantity, 910 cc, sp gr, 1007, chlorides, 0.25, blood urea, 0.136, albumin, 1.25, sugar, negative, phenolsulphonephthalein elimination, 47

March 6 Quantity, 400 cc, sp gr, 1011, chlorides, 0.25, blood urea, 0.103, albumin, 0.5 Dog does not eat and vomits occasionally Urine sediment contains renal cells but no casts.

March 8 Quantity 300 cc., sp gr, 1008 otherwise as above except blood urea, which is 0.145, and chlorides, 0.10

March 10 Does not eat drinks much water and vomits occasionally Quantity, 850 cc, sp gr, 1008 albumin, 0.75, blood urea 0.300 urine chlorides, 0.10

March 12 Same as above Blood urea, 0.200 phenolsulphonephthalein elimination, 33

March 13 Since the dog was apparently in terminal coma, he was killed Autopsy The right kidney is approximately a fourth smaller than the left The cortex is anemic and the pyramids congested The left kidney appears normal grossly No other gross pathology was evident Histology Right kidney Many hyaline casts are in the tubules of the cortex. There are many more in the tubules of the medulla. A few glomeruli show slight hyaline degeneration. There are many small areas of small lymphocytic infiltration in the cortex chiefly located near and about the glomeruli and blood vessels The walls of the blood vessels are thickened and thrombi partially occlude some of the smaller arteries Left kidney Same changes are present as are described in right kidney but not so extensively Thin



Fig 5—Microphotograph from the right kidney of Dog 6 showing hyaline material in the glomerular space and lymphocytic infiltration in and about the tubules

paraffin section shows that the tubular epithelial cells are practically nongranular, and that some of them contain aggregates of material which stain similarly to the hyaline casts in the tubules

Protocol Dog 7—Blood vessels of left kidney occluded one hour

March 12 to 29 Preliminary control period on diet Quantity of urine, 320 cc to 450 cc., sp gr, 1025 chlorides, 0.55 to 0.48, blood urea 0.065 to 0.072, no albumin no sugar, phenolsulphonephthalein elimination 75 to 80

March 29 Occluded blood vessels of left kidney for one hour

March 30 Fifteen hours after operation 150 cc urine excreted

March 31 Quantity of urine 150 cc., sp gr 1005 albumin, slightly positive, no sugar, chlorides, 0.30, blood urea, 0.085 phenolsulphonephthalein elimination, 80 Dog does not appear to be as sick as dogs in which both kidney vessels were occluded

April 1 Quantity of urine 200 cc., sp gr 1020 albumin, 0.20 no sugar, chlorides 0.32, phenolsulphonephthalein elimination 70

April 2 Quantity of urine excreted, 350 cc. sp gr 1025, albumin, 0.20, no sugar chlorides, 0.32, phenolsulphonephthalein elimination 70

April 3 to 8 Quantity of urine excreted 410 to 425 cc.

April 8 Quantity of urine excreted, 450 cc, sp gr, 1025, albumin, slightly positive, no sugar, chlorides, 0.9, blood urea, 0.035, phenolsulphonaphthalein elimination, 73

April 8 to 28 Quantity of urine excreted, 425 to 450 cc, no albumin

Oct 1 Killed Left kidney one third normal size No histologic examination made

Protocol, Dog 8—Blood vessels of both kidneys occluded for one half an hour

April 24 to May 5 Preliminary control period on diet Quantity of urine, 310 to 450 cc, sp gr, 1025 to 1030, albumin, and sugar negative, chlorides, 0.81 to 0.92, blood urea, 0.040 to 0.048, phenolsulphonaphthalein elimination, 72 to 81 Weight, 9.8 kg

May 5 Blood vessels of both kidneys occluded for one half hour

May 6 Quantity of urine, 195 cc, sp gr, 1020, albumin, 0.25, no sugar, chlorides, 0.37, blood urea, 0.045, phenolsulphonaphthalein elimination, 65 Dog is depressed, does not eat, and vomits occasionally

May 7 Quantity of urine, 217 cc, sp gr, 1018, albumin, 0.5, no sugar, chlorides, 0.81, blood urea, 0.048, phenolsulphonaphthalein elimination, 77 Dog still depressed and vomits occasionally

May 8 to 11 Quantity of urine, 210 to 250 cc

May 12 Quantity of urine, 250 cc, sp gr, 1025, albumin, 0.25, no sugar, chlorides, 1.0, blood urea, 0.045, phenolsulphonaphthalein elimination, 73

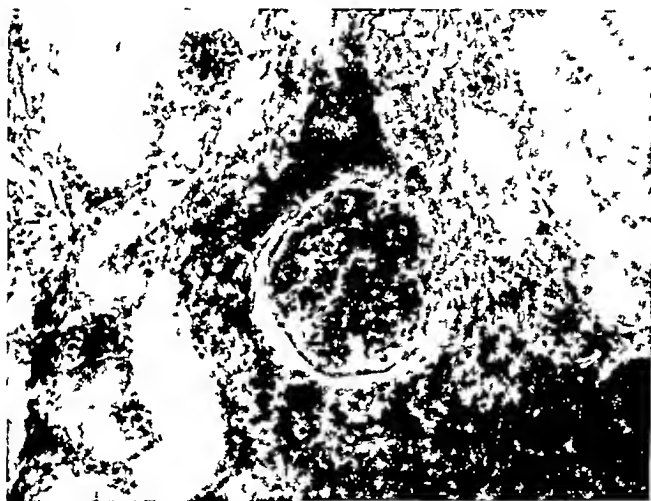


Fig 6—Microphotograph of the kidney of Dog 8 showing an increase in connective tissue and lymphocytic infiltration about the glomerulus.

May 14 to 19 Quantity of urine, 240 to 250 cc, sp gr, 1022 to 1040, albumin and sugar, negative

Aug 20 Quantity of urine, 300 cc, sp gr, 1040, albumin, 1.0, no sugar, blood urea, 0.018, chlorides, 0.87, phenolsulphonaphthalein elimination, 59

July 29 Dog was operated and Pawlow pouch made

Aug 21 to Sept 5 Dog showed albumin in urine, 0.5 to 1

Sept 11 Quantity of urine, 290 cc, sp gr, 1035, albumin, 0.5, no sugar, chlorides, 1.6, blood urea, 0.025, phenolsulphonaphthalein elimination, 66

Oct 1 Killed. Autopsy Changes not marked Kidneys appear to be slightly smaller than normal and are scarred. Histologic examination reveals only small areas of connective tissue in the medullae and a few glomerular spaces that contain a hyaline material There is an increase in connective tissue about many of the glomeruli

Protocol, Dog 9—Blood vessels of both kidneys occluded for one hour

May 19 to May 26 Preliminary control period on the diet Quantity of urine excreted, 250 to 275 cc, sp gr, 1025, albumin and sugar, negative, chlorides, 0.48 to 1.2, blood urea, 0.042 to 0.057, phenolsulphonaphthalein elimination, 86 to 90

May 27 Blood vessels of both kidneys occluded one hour Weight, 81 kg

May 28 Eighteen hours after operation Catheterized specimen, 22 c.c., sp gr 1015, albumin, 15, no sugar, chlorides, 0.2, blood urea, 0.113 phenolsulphonephthalein elimination, 22 Dog is depressed, vomits, and does not eat

May 29 to 31 Thirty c.c. obtained by catheter sp gr, 1015, albumin, 11, no sugar chlorides, 0.4, blood urea, 0.033, phenolsulphonephthalein elimination, 50

June 1 to 7 Dog has lost weight (76 kg) Quantity of urine excreted, 210 to 320 c.c., phenolsulphonephthalein elimination, 65

June 19 Quantity of urine excreted, 300 c.c. sp gr, 1010, albumin and sugar, negative, chlorides, 0.51, blood urea, 0.035, phenolsulphonephthalein elimination, 38

June 20 to July 23 About the same as above without notable change Quantity of urine, 325 c.c., sp gr, 1010, albumin and sugar, negative chlorides, 0.55, blood urea, 0.035, phenolsulphonephthalein elimination, 42

July 28 Quantity of urine, 210 c.c., sp gr 1018 albumin, slight trace no sugar, chlorides, 0.5, blood urea, 0.03, phenolsulphonephthalein elimination, 54

Aug 20 Quantity of urine, 275 c.c., sp gr, 1021, albumin and sugar, negative chlorides, 0.73, blood urea, 0.038 phenolsulphonephthalein elimination, 43



Fig 7—Microphotograph of the right kidney of Dog 9 showing hyaline material in the tubules and some of the glomerular spaces. In areas the tubules and hyaline material stain deeply blue suggesting calcium deposition

Aug 26 Blood vessels of both kidneys clamped a second time for one half an hour On opening abdomen, dense adhesions had formed about kidneys and they were a great deal smaller in size

Aug 27 Eighteen hours after occlusion, anuria. Catheterized 12 c.c., sp gr, 1023 albumin, 10, no sugar, chlorides, 0.20 blood urea, 0.051 phenolsulphonephthalein elimination, 27 Dog depressed, does not eat, and vomits occasionally

Sept 3 Quantity of urine excreted, 255 c.c. sp gr 1030, albumin 10 no sugar chlorides, 0.25, blood urea, 0.053, phenolsulphonephthalein elimination 32 Dog does not eat very much food.

Sept. 11 Quantity of urine excreted, 285 c.c., sp gr 1023, albumin and sugar, negative, chlorides, 0.56, blood urea, 0.046 phenolsulphonephthalein elimination 41

Oct 1 Killed. Autopsy Both kidneys scarred, white and contracted the right kidney being more so than the left Histology Right kidney There are extensive areas in the cortex in which there is great destruction of the parenchymatous tissue Many of the glomeruli are replaced by a clear hyaline material and are crowded together so that ten or more glomeruli come into contact with one another In these areas as well as in the tubules of the medulla there are many hyaline casts In the cortex in the areas that show much degenera

tion, there are small clumps of material that stain deeply blue, suggesting calcification. There is an increase of connective tissue about a few of the glomeruli and in the medulla. Left kidney. The same condition is present as described above in the right kidney, but not so extensively.

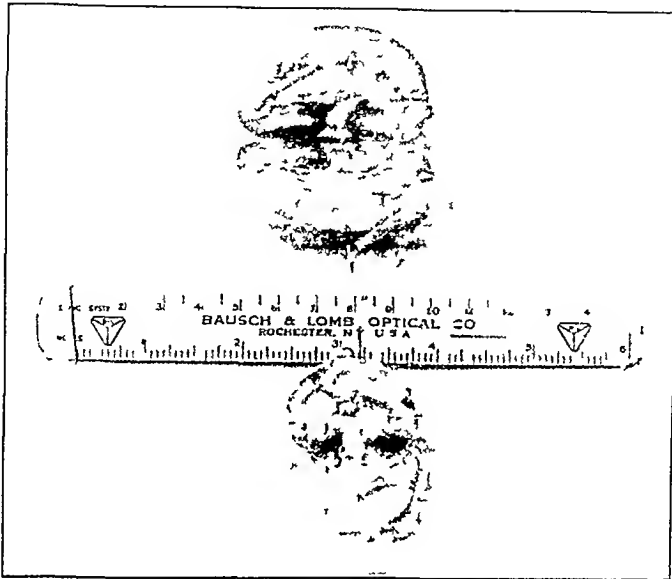


Fig 8—Kidneys taken from Dog 9 that had the circulation of both kidneys occluded for sixty minutes

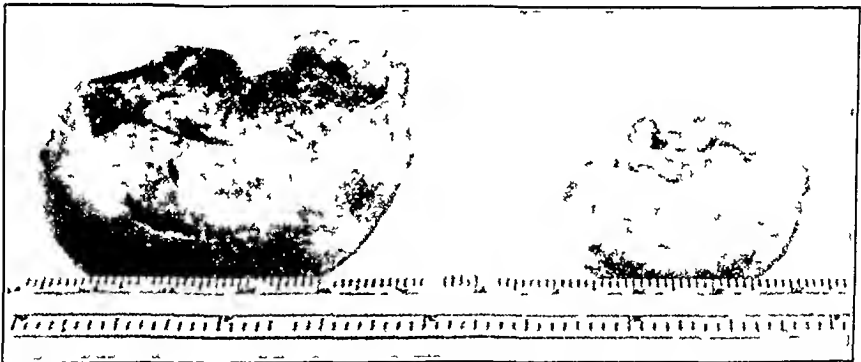


Fig 9—Kidneys taken from Dog 2 that had the circulation of both kidneys occluded for thirty minutes

In the second group of twelve dogs, in which the kidney vessels were clamped for forty-five minutes, all survived the operation with the exception of two that died during the second week following the operation. Some of these dogs showed marked symptoms of anorexia and vomiting, lasting from four to five days, others, only mild symptoms lasting one or two days. In the first twenty-four hours it was almost always necessary to catheterize in order to obtain a sample of urine. Albumin was present in the urine from two weeks to a month following the operation. Many renal cells were present in the urine, but casts were few and were found with difficulty. Sugar

was examined for, but was never found. The normal elimination of phenol sulphonephthalein in this group varied from 70 to 90 per cent. The second day after the operation, elimination varied from 16 to 30 per cent, the seventh to fourteenth day, it varied from 50 to 70 per cent. No return to normal was observed. Anatomically one kidney was always apparently affected more than the other. In one case the left kidney weighed 6.5 gm, the other 2.2 gm. Also some dogs showed greater atrophic changes than others.

SUMMARY OF RESULTS

A dog may or may not survive clamping of the kidney vessels for periods of from thirty to sixty minutes. Whether or not survival occurs depends upon unknown factors governing the susceptibility of the animal to such a procedure. Physiologic and anatomic changes occur even after an ischemia of thirty minutes, one out of two dogs with such treatment, dying twenty seven days after the operation. Eleven out of thirteen dogs survived the forty five minute ischemia of the kidneys for indefinite periods. Two out of four dogs survived the sixty minute ischemia for indefinite periods the other two survived fourteen and sixteen days.

The chief objective symptoms following the ischemia of the kidneys were anorexia, vomiting and asthenia. The symptoms were more marked following an ischemia of sixty minutes than of a shorter period. One of the animals that survived manifested these symptoms at intervals for a month after the operation. The symptoms were most severe for the first three or four days except in those animals that died within two weeks after the operation, in which the symptoms continued without abatement until death. Three animals died in coma.

The blood urea was increased following the operation in every case except in Dog 8. In Dog 7 there was only a slight increase in the blood urea twelve hours after the clamping of the vessels of one kidney for four hours. In the dogs that did not survive the operation the blood urea increased up to the time of death, being as high as 0.300 gm in one. In those that survived, the blood urea returned to normal as the objective symptoms subsided.

No urine was voluntarily passed during the first twenty four hours after the operation. However, at this time (eighteen to twenty four hours) from 30 to 60 cc of urine could usually be obtained by catheter. Catheterization at twelve hours, in every case that it was done either failed to demonstrate the presence of urine in the bladder or resulted in obtaining amounts less than 5 cc. Dog 7 must be excepted but in this animal the vessels of only one kidney were clamped.

The permanent effect of the ischemia on the quantity of urine was variable and no generalization can be made.

The urine chlorides were decreased in the majority of cases no change occurring in Dog 7 and only a temporary decrease in Dog 8.

No noteworthy change was observed in the specific gravity of the urine. Albumin a few red cells and many kidney cells were present in the urine during the first week. The red cells usually were absent after twenty four hours. Kidney cells persisted for from five to twelve days. Albumin

was present for from three weeks to four months. In one dog an operation after the disappearance of albumin from the urine caused it to reappear. Casts could be found only with difficulty.

Although we followed the total nitrogen and the urea of the urine, the results were so variable because of the vomiting, anorexia, etc., that they proved nothing. The results in general suggested what might be predicted, that during the period following the operation, in which the animal showed symptoms and a high blood urea, there was a decreased elimination of urea and total nitrogen.

During the time that the kidney vessels were occluded, the kidney would swell and become dark in color. On releasing the clamps, the normal color of the kidney would slowly return, appearing normal at least throughout the visible surface of the kidney.

Anatomic changes of the kidney occurred after a thirty-minute occlusion of the blood vessels of the kidney, but the changes were more extensive following a sixty-minute occlusion. Both kidneys were not usually injured to the same degree and different portions of the same kidney were not affected alike. In most sections, the inner portion of the cortex manifested more injury than the subcapsular portion. It was impossible for us to arrive at a generalization as to whether or not the tubular epithelium was affected more than the glomerular. The results show that anatomically, the occlusion of the blood vessels of the kidney results in a degenerative process that leads to what is commonly called a "small white kidney."

DISCUSSION

To us, one of the most striking findings in these results is the marked difference in the reaction of the kidneys of the same animal to the identical procedure. Since we are certain that the clamps did not slip, that no permanent injury of the renal artery or vein resulted from the clamping, and that it was not the same kidney in different dogs that showed the most atrophy, the only way we can account for this difference is, to assume that the collateral circulation of the least injured kidney was superior to that of the other kidney.

In a general way the effect of ischemia on the anatomy of the kidney as observed by us, confirms the observations of Guthrie, and of Eisendialth and Strauss, and is to be expected in view of the effect of prolonged ischemia on parenchymatous tissues in general. We were surprised to find that a sufficient amount of kidney tissue frequently survived a sixty-minute ischemia to maintain the animal in a normal condition for five months after the acute symptoms disappeared. Our observations of the development of what apparently appears to be a secondarily contracted kidney, directs attention to the fact that such a process occurred in our experiments independent of systemic infection. Thus the changes are to be accounted for purely by the ischemia, which may be further prolonged by a "postanemic" spasm following removal of the clamps (Carlson and Stoll). Can these observations be related to the view of Volhard¹¹, that the changes in acute nephritis are due to an ischemia produced by a toxic agent that causes spasm of the *vas afferens*?

It is obvious from our results that a picture simulating acute parenchymatous nephritis can be produced in the dog by occluding the blood vessels of the kidney for thirty minutes or more. Hence, it is possible for us to study the effect of such a nephritis on gastric secretion.

Our results further show that in operations on the kidney neither clamps nor forced traction should be applied to the kidney pedicle for prolonged periods of time—certainly not longer than thirty minutes. Our observations may account for some of the small white kidneys found at autopsy, or by a second operation, in those cases in which the kidney had been previously operated upon.

SUMMARY

Ischemia of the kidney for periods of from thirty to sixty minutes in the dog causes degenerative changes that frequently result in the formation of a "small white kidney." Both kidneys are usually not affected to the same extent, the most probable explanation for the difference being a variability in the amount of collateral circulation. Dogs may or may not survive a thirty to sixty minute period of ischemia of both kidneys. The majority, however, survive an ischemia of forty five minutes. The symptoms that result from such a procedure are vomiting, anorexia, asthenia and auria. Albumin and cells (kidney cells, red and white blood cells) are present in the urine for varying periods of time. Albumin persists for from one to four months. The blood urea is always increased, and in the cases in which a severe reaction occurs, it may be as high as 0.300 gm. We believe that this method for the production of nephritis is most satisfactory for the study of the effect of nephritis on gastric secretion or on other physiologic processes, in which it is necessary to avoid the use of toxins or chemicals, which per se might complicate the result.

REFERENCES

- ¹Bradford. *Lancet*, 1904, cxviii, 191
- ²Foa. Quoted by MacNider
- ³Eisendrath and Strauss. *Jour Am Med Assn* 1910 ix, 2286
- ⁴Guthrie. *Arch Int Med*, 1910, v, 232
- ⁵Litten. *Virch Arch*, 1881 lxxviii, 508
- ⁶MacNider. *Jour Med. Research* 1911, cxiv, 424
- ⁷Marshall and Crane. *Am Jour Physiol* 1923 lxi, 387
- ⁸Rowntree, Fitz and Geraghty. *Arch Int Med* 1913 xi, 121 1912 ix, 234
- ⁹Stoll and Carlson. *Am Jour Physiol* 1923, lxvii, 103
- ¹⁰Thorel. *Virch Arch* 1896 cxlv, 297
- ¹¹Volhard. *Die Brightsche Nieren Krankheiten* Berlin 1916

STUDIES ON NEPHRITIS^{*}

II GASTRIC SECRETION IN NEPHRITIS

BY E T McENERY, M S, M D, JACOB MEYER, M D, AND A C IVY, M D
CHICAGO, ILL

THE occurrence of gastrointestinal disturbances in the course of nephritis is well recognized by clinicians. The symptoms have been ascribed to an edema of the gastric mucosa, and also to the influence of the uremic condition on the central nervous system. Von Noorden¹ suggests, a greater part of the symptoms are due to the action of toxins on the gastrointestinal mucosa. In cases of anuria, numerous observers¹ have noted that substances usually excreted by the kidney are eliminated by the alimentary tract. Of these substances, the most irritating is ammonia, which is formed in the intestine by the decomposition of the urea that is eliminated. The feces in uremic diarrhea contain much ammonia.

Several writers have reported the results of gastric analysis in cases of acute and chronic nephritis with edema and in the acute relapses of interstitial nephritis. Biernacki² found that the secretion of HCl, rennin and pepsin to be diminished. Von Jaksch³ noted a deficiency in HCl. Von Noorden¹ found an excess of HCl after meals in four out of nine patients with acute nephritis. Kiakow⁴ observed that in twenty-six cases of diffuse nephritis HCl was never absent and was diminished in eight cases. Zipkin⁵ recorded similar results in twenty-three cases. One of Von Noorden's assistants (M Dapper) studied gastric secretion in fifteen renal cases. In three, there was an absence of free HCl, in seven it was diminished, and in the others it was normal.

We have sought to determine the changes that occur in the secretion of gastric juice in cases of nephritis in man and in Pawlow pouch dogs on the production of an experimental nephritis without the use of toxic agents.

METHOD

In our experiments on animals Pawlow pouch dogs were used. The nephritis was produced by occluding the blood vessels of the kidney for various periods of time.

Van Slyke and Cullen's modification of Marshall's urease method was used for determining blood urea. The same method was used for determining the urea of the gastric juice, the free acidity of the gastric juice being neutralized completely and the combined acidity in part, so that the acidity was not more than 0.009 per cent. We always set up two tubes, one (a) with gastric juice plus enzyme, the other (b) with gastric juice without

^{*}Received for publication July 10 1926

From the Department of Physiology, the University of Chicago and Northwestern University Medical School

enzyme We subtracted the latter result from the result obtained in 'a' in order to arrive at a correct figure for urea. This was necessary because gastric juice contains ammonia

EXPERIMENTAL OBSERVATIONS

The Effect of Experimental Nephritis on Gastric Secretion—Two Pawlow pouch animals were studied. After a preliminary control period, during which the gastric secretory response to a standard meal and 1 mg of histamine was determined, the blood vessels of the kidneys were clamped forty five minutes, a procedure that causes nephritis.² Following the clamping of the kidney vessels, the blood and urine were studied simultaneously with the gastric response to a meal and to 1 mg of histamine.

It is quite clear from the results shown in Table I that the type of acute nephritis produced by our procedure decreases, but does not abolish, the

TABLE I

THE EFFECT OF NEPHRITIS CAUSED BY OCCLUSION OF THE BLOOD VESSELS OF THE KIDNEY ON GASTRIC SECRETION
PAWLOW POUCH DOGS

DOG	METHOD OF EXCRETINO OLANDS	BEFORE 1 KIDNEY INJURY OASTRIC SECRETION			AFTER KIDNEY INJURY OASTRIC SECRETION			REMARKS
		AMT C C	FREE ACID	TOTAL ACID	AMT C C	FREE ACID	TOTAL ACID	
Dog 3	Meal	.5	87	110	11	37	76	Dye elim 34% Blood urea, 0.141 gm
	Histamine	16	80	90	1	46	87	
Dog 9	Meal	1	51	75	11	21	37	Dye elim 38% Blood urea, 0.112 gm.
	Histamine	11	40	85	6	35	71	
		SECOND CLAMPING						
	Meal	12	10	9	10	60	78	Dye elim 32% Blood urea, 0.053 gm
	Histamine	18	40	65	11	30	57	

Histamine secretion collected for two hours

Meal secretion collected for two hours Acidity expressed in clinical units

Results recorded before kidney injury are averages of at least ten meal responses and two histamine responses.

After kidney clamping we give typical experiments as the dog would not eat at all times. The histamine responses are averages. Normal dye elimination in these dogs was from 85 to 90 per cent.

TABLE II

EFFECT OF INCREASED BLOOD UREA ON THE UREA CONTENT OF GASTRIC JUICE
PAWLOW POUCH DOGS

DOG	BEFORE KIDNEY INJURY		AFTER KIDNEY INJURY		REMARKS
	UREA IN BLOOD	UREA IN GASTRIC JUICE	UREA IN BLOOD	UREA IN GASTRIC JUICE	
Dog 9	0.057		0.113	0.060	
	0.054		0.114	0.132	
			0.035	0.012	After recovery
			0.038	0.014	
			0.050	0.020	Clamped kidneys again ½ hr
			0.052	0.024	
Dog 3			0.046	0.017	
	0.024	0.010	0.030	0.015	
	0.07	0.011	0.080	0.026	Death
			0.141	0.039	

TABLE III

EFFECT OF INTRAVENOUS INJECTION OF UREA ON THE UREA CONTENT OF GASTRIC JUICE
PAWLOW POUCH DOGS

	UREA IN JUICE BEFORE INJECTION	UREA IN JUICE AFTER INJECTION	REMARKS
Dog 1	10 mg	92 mg *	Secretion stimulated by histamine
Dog 2	2 mg	69 mg	Secretion stimulated by histamine
Dog 3	10 mg	20 mg	Secretion stimulated by histamine
Dog 4	0 mg	2 mg 19 mg 7 mg 5 mg	5 min after 10 " " 15 " " 20 " " Secretion stimulated by histamine

Two grams of urea in 20 cc of normal salt solution were injected intravenously. The urea in the juice is expressed in milligrams per 100 cc of juice. Dog 4 had a pouch of the entire stomach which made it possible for us to collect large quantities of secretion at five minute intervals. Repeated this experiment four times with similar results the highest concentration appearing during the 10 to 20 minute period.

*Nephritis had been produced in this animal by clamping the kidney vessels two months previous to this experiment. Dogs 2, 3 and 4 were normal.

secretory response to a meal. Only typical results are shown in the table of the response when the animal ate the entire meal. As recovery occurred the response gradually returned to normal (Dog 9). No permanent damage of the gastric glands resulted.

Histamine was used to determine whether or not the gastric glands were fundamentally intact. This procedure could be and was used when the animal refused food. Both animals when they were sick, i.e., manifested anorexia, some vomiting, and a high blood urea, responded to histamine, but not normally, the amount of secretion being decreased more than the acidity. As recovery occurred the response returned to normal. This further shows that no permanent damage of the gastric glands resulted.

Urea in Gastric Secretion.—While ascertaining the effect of intravenously injected urea per se on gastric secretion, which is negative in the doses used, it was found that small amounts of urea (0 to 10 mg) are present normally in the secretion of a Pawlow pouch. We then decided to ascertain if any quantitative relationship existed between the urea concentration of the blood and gastric secretion.

The results shown in Tables II and III show that the higher the concentration of urea in the blood, the higher the concentration in the gastric juice.

Although it would be very interesting to find whether or not a definite ratio exists, we have not as yet attempted to answer this question. Many more observations than we have made would be necessary to establish such a ratio. It is interesting to note (Table III) in this connection that the animal in which most urea was eliminated in the gastric secretion was Dog 1 with experimental nephritis. The result on Dog 4 in Table III suggests that there is a latent period of about five minutes before elimination by the gastric mucosa is initiated.

TABLE IV
GASTRIC SECRETION IN PATIENTS WITH NEPHRITIS
LACTOSE TEA MEAL

PATIENT	GASTRIC SECRETION		REMARKS
	FREE ACID	TOTAL ACID	
Case 1 Mrs L G Chronic interstitial nephritis Non N Salt free diet	trace	20	N.P.N -53.2 mg Urea N-25.8 mg Creatinine-1.3 mg Chloride 509 mg
Case 2 Mrs R, age 59 Chronic interstitial nephritis with hypertension	20	25	
Case 3 Mrs D Chronic nephritis	25	30	
Case 4 Chas K Nephritis with edema Chronic Age 18	45	65	N.P.N -103.1 mg Urea N-122 mg Uric acid-6.2 mg Creatinine 5.4 mg Patient died, uremia
Case 5 Age 13 Acute nephritis with edema and uremic vomiting	35 25 18	75 30 28	Blood pressure 20+ systolic Patient died Analysis of vomitus
Case 6 R B Age 14 Chronic nephritis with edema	16	24	N.P.N -57 mg Urea N-32 mg Creatinine -2 mg Chlorides -717 mg Urine albumin -0.6%

Patients from the service of Drs A Edwards and Solomon Strouse Michael Reese Hospital Chicago

CLINICAL OBSERVATIONS

The Effect of Nephritis on Gastric Secretion—Eighteen cases of nephritis have been studied

In six cases (Table IV) the response to a lactose tea meal was determined. Free acid was found in every case, even in Case 5 with uremic vomiting.

The continuous gastric secretion (not gastric contents) was examined in twelve cases. Free acid was present in three of the cases (see Tables V and VI), it being as high as 80 clinical units in a patient that died several days later because of the severity of his nephritis.

The gastric response to histamine was observed in three cases (Table VI). An increase in the quantity of secretion occurred in every test. An increase in acidity occurred in every test except one (FS). Two days later, however, in the same patient under practically the same conditions histamine caused an increase in acidity.

Urea in the Gastric Secretion of Patients with Nephritis—It should be pointed out that in collecting gastric secretion for these analyses the stomach was emptied and the contents discarded. The patient was then instructed not to swallow his saliva, the secretion was then collected for half an hour, and kept under toluol for about four hours before it was possible to examine it. Because of the possibility of the contamination of the gastric secretion with salivary urea, in spite of our attempts to prevent it the results cannot be considered as exact as the results on Pawlow pouch dogs.

Our findings in patients (Tables V and VI) approximately parallel our findings on dogs (Table II), the higher the blood urea concentration the

TABLE V

CONTINUOUS GASTRIC SECRETION IN NEPHRITIS AND THE AMMONIA AND UREA CONCENTRATION OF THE GASTRIC JUICE

PATIENTS

PATIENTS	GASTRIC JUICE		NH ₄ MG PER 100 C C	UREA MG PER 100 C C	URINE AND BLOOD
	FREE	TOTAL			
D Mc L Age 32 Chronic nephritis Hyper tension	32 15	60 27	42 —	4 4	Albumin and casts Blood urea $\sqrt{125}$ Creatinine -4.5
A Gr Age 32 Chronic nephritis Hyper tension Pericarditis with effu sion Died	0	25	70	20	Albumin -0 No casts Blood urea -280 Creatinine -12.0
B R Malignancy of prostate with retention	0	20	Nothing but mucus		Blood urea -150 mg per 100 c c
O M Chronic nephritis Chronic myocarditis with decompensation	0	15	9	10	Blood urea -37 Creatinine -1.5 Albumin ++++ Casts and cells
M C Acute exacerbation of chronic nephritis with hypertension	0	22	40	4	Blood urea -64 Creatinine -2.0 Albumin ++++ Casts -
J B Age 34 Acute nephritis with hypertension	0	22	22	5	Blood urea -60 Creatinine -1.8 Albumin ++++ Casts Blood ++++
A M Age 63 Chronic nephritis with uremia and hyperten sion	0	35	33	27	Blood urea -150 Creatinine -3.0 Albumin -+++
Wm Cy Age 32 Chronic nephritis	0	7	Not enough to examine		Blood urea -80 Creatinine -2.4 Albumin ++++
M B Chronic nephritic	0	27			Blood urea -124

Cases for Cook County Hospital Chicago

higher the concentration of urea in the gastric juice. The injection of histamine in four out of five tests increased the concentration, and hence the elimination, of urea in the gastric secretion.

The ammonia content of gastric secretion of these patients with nephritis is in most instances definitely greater than normal, the normal content being rarely more than 5 mg per 100 c c of juice.

DISCUSSION

In considering our results it is necessary to keep in mind the experimental method used by us to produce nephritis and that the results obtained by this method might not be comparable to conditions met with in man. Our experimental observations show a decrease in gastric secretion during the acute manifestations of nephritis, which is readily explained by vomiting (dehydration), anorexia, toxemia, and nervous inhibition due to distress. This is certainly what one would expect to occur in acute nephritis in man. But it happened that the single case of acute nephritis reported in our group of patients (Table IV) showed normal acid values in the vomitus expelled

during uremic vomiting. This possibly is an exceptional case, but it shows that at least in some cases of uremia the gastric mucosa can still form gastric juice. We cannot say to what extent, but it is probably less than normal, judging from our experimental results.

The occurrence of free acid in the continuous secretion of only three out of twelve cases of chronic nephritis twelve hours after the last meal is

TABLE VI

SHOWING GASTRIC SECRETORY RESPONSE TO HISTAMINE AND THE AMMONIA AND UREA CONTENT OF GASTRIC SECRETION IN PATIENTS WITH NEPHRITIS

PATIENTS	BEFORE HISTAMINE					AFTER HISTAMINE					REMARKS
	AMT IN C C	FREE ACID	TOTAL ACID	NH ₃ MO PER 100 C C	UREA MO PER 100 C C	AMT IN C C	FREE ACID	TOTAL ACID	NH ₃ MO PER 100 C C	UREA MO PER 100 C C	
M C (1) Acute exacerbation of chronic nephritis with hypertension Blood urea -720 Creatinine -2.8 Albumin and casts	10	0	15	7.6	18	30	50	60	11.2	18	Bl Pr 190 110
D D (2) Chronic diffuse nephritis with hypertension Blood urea -860 Creatinine -2.1 Albumin and casts	12	80	122	6.6	36	60	137	150	-	43	Bl Pr 198 120
F S (3) Chronic nephritis Blood urea -225 Creatinine -4.6 Albumin and casts	6	40	70	41	17	Trace of bile present					Bl Pr 152 76
D D (2) Chronic nephritis Blood urea -221 Creatinine -5.5	10	0	35	60	20	40*	42	72	48	46	
F S (3) Chronic nephritis Blood urea -960 Creatinine -3.7 Died three days later	20	32	70	2	4	80	50	100	2	14	
F S (3) Blood urea -317.0	10	0	30	81	102	60	25	30	72	144	

Simple of gastric contents 30 minutes after histamine Stomach was not emptied Thirty minutes continuous secretion.

Sample of gastric contents 20 minutes after histamine. Stomach was not emptied. Thirty minutes continuous secretion.

unusual, since free acid is present in the continuous secretion of over 80 per cent of normal individuals. This demonstrates that there is some depression of the gastric glands in chronic nephritis as it occurs in man. However, the gastric glands in the six cases shown in Table IV were still sufficiently intact to respond to a lactose-tea meal. It is interesting to note that the three cases with edema responded to the lactose-tea meal and that in two of these the response was quite normal. We wonder if this observation might not be significantly related to the fact that any procedure that tends to produce a hydremia augments gastric secretion.

Since we have found that urea is present in small amounts in pure gastric juice of normal animals, it is not surprising to find that its concentration in gastric juice is increased by any procedure that raises its concentration in the blood.

Chaillot and Canti⁶ have reported that urea occurs in the vomitus of patients with nephritis. Since urea is present in saliva,⁷ the significance of their observation is questioned. Our findings on dogs with isolated pouches show without question that in nephritis we should expect and do get an increase in the urea content of gastric juice.

Our observations have an obvious bearing on what has been termed vicarious elimination^{1, 8} and show that the stomach, as well as other portions of the gastrointestinal tract, must be considered as one of the eliminating organs in cases of nephritis and anuria.

The high ammonia content of the gastric juice in the patients with nephritis might come from the normal source of the ammonia in gastric secretion, which is unknown, or from bacterial decomposition of the urea in the absence of free acid, provided bacteria cannot decompose urea in the presence of free acid.

SUMMARY

1 Acute nephritis produced in dogs by occlusion of the kidney vessels for forty-five minutes decreases, but does not abolish, the gastric secretory response to a meal and to 1 mg. of histamine.

2 The chronic nephritis that follows such a procedure does not appreciably affect gastric secretion.

3 Urea (2 to 10 mg. per 100 c.c.) in small amounts has been found in the gastric secretion of most of the normal dogs studied.

4 The intravenous injection of urea causes an increase in the urea in the gastric juice, and associated with the increase in blood urea in experimental nephritis there is an increase in the urea in the gastric juice.

5 Although the gastric glands in nephritis are probably somewhat depressed as judged from the continuous secretion, it is clear from our results that they will respond to a lactose-tea meal or to an injection of histamine. We believe, however, that exceptions to this general statement may be found.

6 We have found urea in the saliva and bile free gastric juice of patients with nephritis, the urea content of the juice being high usually in those cases that had a high blood urea content.

*We have also found urea in bile.

7 We believe that these observations have a significant bearing on the role that so called vicarious elimination plays in nephritis and anuria

REFERENCES

- ¹Von Noorden Metabolism and Practical Medicine, Uremia, 1907, 11 436 438, 506
- ²Biernacki Zentralbl f klin Med, 1890 265, Berl klin. Wchnschr, 1891, pp 611, 65.
- ³Von Jaksch Uramie in Enlenburgs Encyklop 1890 xxi 90
- ⁴Von Noorden Metabolism and Practical Medicine, Vienna 1907, 11 437
- ⁵Krakow Loc cit. Von Noorden
- ⁶Zipkin Loc cit, Von Noorden
- ⁷Charcot and Cantl Cited by W A Myers Jour Med Assn, 1926 lxxxvi, 1198
- ⁸Hench and Aldrich Jour Am Med Assn 1923 lxxxi, 199
- ⁹Myers Jour Am Med Assn 1926 lxxxvi 1198
- ¹⁰McEnery, Meyer and Ivy Jour Lab and Clin Med 1927, xii, 349, Article I

METHODS FOR PREVENTING THE AGGLUTINATION OF BLOOD BY GLUCOSE SOLUTIONS*

BY WALTER R. PENDLETON S.B., CHICAGO, ILL

I OBSERVED that glucose solutions may cause marked and prompt agglutination of human blood in vitro. I have been unable to find reference to such a phenomenon in discussions of glucose therapy, but do find that it is already known among certain laboratory men.¹ I wish to present some characteristics of the phenomenon, and to give simple methods for inhibiting or preventing its occurrence. My work has been carried out entirely in vitro and so such statements as I make are given with full realization of the need for still further investigation.

Williams and Swett describe glucose reactions occurring within a few minutes to one half hour after glucose injection and ordinarily passing off within twenty four hours, the patient suffering from chill, fever and marked prostration. It is a question whether blood agglutination might not play a part in such a series of symptoms. Williams and Swett stated that glucose solutions become acid on autoclaving and that this might be the cause of the reaction. They found that if the P_H of the glucose solutions be brought to approximately 7.4 with a potassium phosphate buffer there would be no reaction. Ten per cent glucose solutions were used.

Stoddard² observed similar glucose reactions which he indicated to be by no means unusual. He stated that no further reactions occurred in the Massachusetts General Hospital during the eight months that had elapsed since they began using a sodium phosphate buffer in the glucose solutions. But other refinements were also simultaneously made in the glucose therapy. He doubted that there would be enough acid in glucose solutions to cause an acidosis reaction. Stoddard stated that 500 cc of the most acid solution he obtained, P_H 4.4, would probably not cause a greater drop than 0.06 in the blood P_H . His attitude is in accord with observations by Seibert³ who

found that rabbits give no reaction on injecting 5 cc of sterile distilled water of P_H 4.6 Stoddard used 5 per cent glucose solutions

Glucose solutions were prepared for the following experiments by making Merck "Pure" glucose to 50 per cent or higher concentration with distilled water. Heat was applied only for the purpose of bringing about solution of the glucose. Portions of the freshly prepared solution were diluted in distilled water as desired. Tests were made with my own blood. In each test whole blood was used immediately after leaving a skin puncture. Glucose solutions and blood were measured in the stem of a hemocytometer white cell pipette and emptied into the concave portion of a hollow ground slide. The mixture was made to the same volume in each test (about 0.016 cc). Air was blown through the pipette onto the drop long enough to mix the materials (2 to 3 seconds). A cover-slip was placed over the drop.

The agglutinating power of the following glucose solutions was tested 5, 7.5, 10, 15, 20, 30, 40, 50, 80, and 100 per cent. One part of blood was used with four parts of glucose solution. Tests showed that this volumetric ratio (of blood to glucose solution) gives roughly the maximum agglutination with solutions of varied concentrations. Solutions containing 5 to 10 per cent of glucose produced decidedly the most marked gross and microscopic agglutination. Agglutination was progressively decreased with each solution of higher concentration. There was no clumping apparent to the naked eye with the 20 per cent solution, but microscopic agglutination was very definite. The 30 per cent solution showed absolutely no gross clumping. The 40, 50, 80, and 100 per cent solutions caused a very loose microscopic agglutination. Thus approximately isotonic solutions (glucose content of 5 to 10 per cent) produced the most marked agglutination.

The next question is what occurs when a glucose solution is mixed with larger quantities of blood as would take place in the passage of the solution into the general circulation? Successive tests were made with 7.5 per cent glucose solutions using a larger proportion of blood each time. With a ratio of blood to glucose solution of 1 to 9 the corpuscles were too much separated by volume of fluid to form large agglutinated masses. Using a ratio of 1 to 4 clumps slightly less than 0.2 mm in diameter formed. They appeared very compact under the microscope. Striking with a teasing needle dissociated practically all clumps, but agglutination was still present microscopically. On standing clumps about 0.1 mm in diameter reformed. With a mixture of equal parts of blood and glucose solution† the corpuscles showed absolutely no tendency to adhere to one another, but during the first minute and a half about 60 per cent of the corpuscles were crenated, for the main part without apparent shrinkage. Crenation was maximum for the series of tests at this point. With a blood to glucose solution ratio of 9 to 1, there was simply a rouleau formation of seemingly normal erythrocytes. Thus on increasing the blood content of a glucose solution in successive steps a cycle occurs in which there is (1) agglutination, (2) absence of agglutination and the appearance of marked crenation, (3) rouleau formation of apparently

*100 cc of solution contained 100 gm of glucose
 †An account of intervening tests is omitted for the sake of brevity.

normal corpuseles. The 40 per cent glucose solution produced a similar cycle but in less distinct steps.

In further tests it was found that a 5 per cent solution of glucose containing 0.2 per cent sodium chloride or sodium hydroxide to N/250 strength (0.016 per cent by weight) would cause no agglutination of blood regardless of the relative amounts of the blood and glucose solutions mixed together. One half this amount of sodium chloride or of sodium hydroxide was not sufficient to prevent agglutination. So less than one tenth as much of the sodium hydroxide (by weight) as of the sodium chloride was required to prevent agglutination. Agglutination by a 40 per cent solution was much inhibited but not prevented by 0.2 per cent sodium chloride and N/250 strength of sodium hydroxide respectively.

Acids increased the agglutination. In each test blood was used with 75 per cent glucose solutions in the ratio of 1 to 4. Hydrochloric acid in N/1000 concentration seemed to cause about three times the agglutination which occurred in an unacidified glucose control. The presence of citric acid in N/50 concentration in a glucose solution converted the blood into quite firm clumps about 0.8 mm in diameter. This was the most marked agglutination obtained in the work on glucose.

A 75 per cent glucose solution with N/50 citric acid content was tested with varied proportions of blood. This gave a cycle almost identical to that obtained with unacidified solutions except for the greatly increased agglutination by the acidified solution at its maximum point. It is especially noteworthy that, regardless of acid content no agglutination persisted when equal parts of blood and glucose solution were well mixed. Buffer substances in the blood would naturally here greatly diminish the activity of the acid.

Tests showed potassium acid phosphate and potassium dibasic phosphate to be antiagglutinating agents as would be expected because of their potassium content. The P_H of N/15 solutions of these salts is 4.49 and 9.18 respectively.⁵ No agglutinating properties remained in 75 per cent glucose solutions after adding 10 to 11 per cent of potassium acid phosphate or 0.1 to 0.2 per cent of potassium dibasic phosphate. Here the dibasic salt was at least five times as effective as the monobasic salt. An N/50 concentration of citric acid necessitated the use of 11 to 12 per cent of sodium chloride or only 0.4 per cent of dibasic potassium phosphate. The buffer agent was much more efficient than sodium chloride in preventing agglutination in an acid medium but in the absence of acids its effect was about the same.

A 5 per cent glucose solution containing 0.1 per cent of oxalic acid (a sufficient amount to prevent clotting) caused a slightly greater agglutination than did the unacidified control. With 0.33 per cent of potassium oxalate in a 5 per cent glucose solution there was no agglutination.

Sucrose and glycerin solutions caused an agglutination of blood similar to that of glucose. Sodium chloride and sodium hydroxide inhibited the agglutination. Citric acid and hydrochloric acid increased agglutination. Magnesium sulphate and ammonium sulphate each prevented agglutination by sucrose solutions.

As to a possible relationship between agglutination and "glucose reactions" it might be pointed out that the solutions used by Williams and Swett

than in others John Hunter was the first to record this observation In the past four years, German and Italian workers have studied the question carefully and feel that the measurement of this phenomenon gives worth while clinical information In the past year, several American writers have substantiated this The rate at which erythrocytes settle when citrated blood is allowed to stand has been used as a clinical laboratory procedure of diagnostic and prognostic worth An increased rate is associated with pregnancy, malignancy, tuberculosis, and acute inflammatory conditions There is the theory of Fahraeus-Hober that this increased agglutination is primarily due to a change in electrical potential between negatively charged erythrocytes and positively charged bodies in the plasma Again there is the theory that the phenomenon is due to an increase in the fibrinogen content

TABLE I

CASE NO	SERIAL NO	TYPE OF CASE	READINGS		
			30 M	60 M	24 HR
1	1959	Married woman, aged 56 Hypertension, constipation, acute tonsillitis, secondary anemia.	90	72	54
2	1965	Man, aged 58 Acute exacerbation of cholecystitis with stones, purpura hemorrhagica of toxic nature, secondary anemia, myocarditis	54	37	33
3	1985	Woman, 28 years of age Chronic cholecystitis Lane's kink, constipation, salpingitis acute	90	70	47
4	2018	Woman, always well until 10 days ago Gastric cancer (early), secondary anemia	87	77	45
5	2021	Woman, aged 30, not feeling well for some time Came for general examination which was negative Small infection on nares which 12 hours later was developed into typical erysipelas	75	54	40
6	2026	Girl, 16 years old Secondary anemia, chronic duodenal obstruction, ileal stasis, ptosis of colon	78	65	45
7	2027	Woman, aged 60 Overweight 25 per cent, chronic cholecystitis, chronic arthritis, acute exacerbation	91	75	50
8	2030	Woman, Afro American, aged 50 Diabetes mellitus in extremis, marked secondary anemia	32	29	28
9	2049	Man, 30 years old Tuberculosis of VIII, IX, X and XI dorsal vertebrae	85	65	44
10	2053	Man, aged 44 Lymphatic leucemia, secondary anemia.	70	60	18
11	2066	Girl, aged 5 years Pulmonary tuberculosis	80	64	57
12	2069	Retroperitoneal and mesenteric lymphosarcoma, ascites	54	41	31
13	2086	Woman, aged 55 Diabetes, nephritis, gallstones with acute exacerbation	72	61	38
14	2106	Man, aged 66 Melanosarcoma with extensive metastasis	86	70	61
15	2107	Woman, 34 years of age Mass at the pylorus (malignant)	88	78	44
16	2108	Man, aged 51 years. Carcinoma of the stomach	91	79	39
17	2113	Woman, aged 58 Secondary anemia, latent jaundice, ulcer of duodenum of 13 years standing with diverticulum, chronic cholecystitis	82	66	38
18	2122	Man, aged 34 Oral and gastric infection with organism of Vincent's angina, secondary anemia, myocarditis	90	75	44
19	2127	Woman, aged 29 Marked secondary anemia, and pregnancy of 2 months	88	78	38
20	2132	Woman, 35 years old Advanced pulmonary tuberculosis	59	39	28

of the plasma It is also known that increase in the sedimentation rate of erythrocytes is accompanied by an increase in globulin

As to the exact mechanism behind this test much remains to be learned It appears, however, fairly clear that the changes occur in the plasma primarily The various theories of how this occurs may be stated and correlated by quoting from Frosch

"Primarily, there is a relative increase of globulin and fibrinogen in certain diseases These particular colloids are composed of larger molecules than the other usual colloids of the blood Thus, there is a diminution in the cohesive power between the molecules making up the plasma Further more, these colloids also have a greater absorption power for the alkaline salts in the plasma and thus the negative electric charge of the red blood cells is diminished, which is conducive to more rapid agglutination of the cellular elements of the blood Briefly, an increase in globulin and fibrinogen produces a lower surface tension of the plasma and a more rapid agglutination of the red cells, and thus a more rapid sedimentation time in those conditions in which these two colloids are increased in the plasma "

Results—In order to conserve space only those cases with a reading below 80 at the end of an hour will be presented in the series of the first one hundred consecutive cases as they were sent to our office by their family physicians for a complete clinical, laboratory, and x ray diagnostic study There are twenty of these in this series

In the second and third series of one hundred cases each, the group which fell below 80 in the first hour is of approximately the same size, viz, 22 in the second series, and 21 in the third series In these two groups, however, we have six cases falling below 80 for which we are unable to account These are recent cases and will be followed up to see what becomes of them

There are several cases in which the test was of the greatest value, for instance, a man, aged sixty two, in whom we were unable to localize a malignancy, although he presented a somewhat cachectic appearance and had a sedimentation rate of 77 59 38 We were so firmly convinced that he had a malignancy that we so reported him and asked for a chance to check up on him In six weeks he developed difficulty in swallowing and we had no difficulty in finding a cancer of the esophagus Again a man, aged fifty three, presented a sedimentation rate of 97 87 42 a filling defect at the pylorus, and a four plus Wassermann and Kahn test Because of the pyloric obstruction and the statement of Brown that 66 per cent of cancer with evidence of gastric cancer and positive Wassermann are cases of gastric cancer, we ignored the sedimentation rate and sent this man to a surgeon A large luetic ulcer was resected We feel now that we would rely upon the sedimentation rate as the differential point Again a highly neurotic man well known to us, came with a pain in the left lower quadrant All tests and examinations were negative except that there was general weakness, a moderate secondary anemia, a rapid sedimentation rate (69 47 20) and a spasm of the colon upon the administration of the first barium enema This was absent at the second examination On the strength of the evidence an exploratory operation was advised but refused for a few weeks He was then studied by another gastro

enterologist who found nothing new except a further development of the secondary anemia. Upon the combined opinions, he submitted to an exploratory operation in about ten days. By this time a mass had appeared in the abdomen. He was found to have an inoperable nonobstructing cancer of the left side of the transverse colon.

Again a woman, aged forty-two, was sent to us by a surgeon to rule the gastrointestinal tract in or out of her diagnostic picture. During the past six months, she had three attacks of chills, fever, and abdominal pain, and diarrhea. Each time she had been attended in a different city and attended by a good physician. In each instance it was thought to be "intestinal flu." When she came to us the last attack had occurred during the previous week. The sedimentation rate was rapid (81-68-35). The gastrointestinal tract was negative. There was a trace of albumin and some pus cells in the urine. These facts compelled us to send her back with a diagnosis of a suppurative lesion of one or both kidneys and a request for a urologic examination. This was done and a right pyonephrosis was found.

The sedimentation rate of the erythrocytes is therefore not a specific test for any one disease but is obtainable in all conditions in which there is accompanying tissue destruction. It has been aptly compared in its use to that of the clinical thermometer. We want to urge its adoption by the internist and by the general practitioner. The internist, because he does not as a rule know his patient intimately, will be given by this test a clue within the first hour of their acquaintance as to whether the patient is the victim of any of the tissue destroying diseases. The general practitioner can employ it with profit because he may have grown deaf to the constant complaining of the neurotic patient who may in the meantime have developed some important pathology.

THE CORPUSCLE VOLUME

The reading at which the red blood cells stands at the end of twenty-four hours is taken as the corpuscle volume.

In the hemolytic anemias the volume index is increased.

Textbooks usually set forth the criteria upon which a diagnosis of pernicious anemia is based as (1) high color index, (2) poikilocytosis, (3) nucleated reds, especially megaloblasts. The variations in hemoglobin readings by different methods and different workers make it a very uncertain procedure in borderline cases unless resort is made to the more complicated analytical methods which are consuming and require too large a quantity of blood. The plus color index, found constantly if our technic is not at fault, in pernicious anemia, is due to the increase in the size of the cell. They are never supersaturated with hemoglobin. The determination of the corpuscle volume is not only more simple and therefore more apt to be correct, but it is a more basic thing and therefore should be used in the study of all anemias. The *volume index* equals $\frac{\text{volume percentage of cells}}{\text{number percentage of cells}}$. A plus volume index is a constant finding in pernicious anemia and is present in early cases in which other qualitative changes are not apparent.

A plus volume index together with the absence of free hydrochloric acid in the gastric juice is practically pathognomonic evidence of pernicious anemia

In the secondary anemias, this information is of value since increase in cell volume must precede the increase in Hb content. Given two patients with low Hb, improvement is necessarily more rapid in the one having the cell volume index nearest normal.

THE ICTERUS INDEX

So far as is known, the yellow color of the blood serum in the *fasting* patient is due to the bilirubin. Increase in color can safely be taken therefore to mean increase in the bilirubin content if the precaution is observed to take the sample in the fasting state. Such increases occur in

1 Hemolytic processes in the body,

such as { Pernicious anemia
Hemolytic jaundice
Hematoma
Rupture of a viscus
Malaria

2 Disturbance in the biliary system

such as { Cholangitis
Cholecystitis
Cholelithiasis
Adhesions about the gall bladder
Diseases of the liver

In dealing with the chronic ambulatory invalid with diseased gall bladder we have not found it of much value because they have come to us in the interval between attacks.

In secondary anemias we find a serum that is paler than normal as a rule.

At the end of the twenty four hour period, after the reading has been taken, the supernatant plasma is pipetted off and compared in a Sahli tube up to the 10 mark with a standard solution of 1/10 000 potassium bichromate. The number of times that the plasma must be diluted to correspond in color with the standard is taken as the *icterus index*. The normal range is from 4 to 6. It has been found that clinical jaundice is always present when the index is above 15, and invariably absent when it is below. The zone of latent jaundice lies, therefore, between 6 and 16.

Latent jaundice is an important bit of information—

1 The prediction of a toxic icterus in

{ Pneumonia
Exophthalmic goiter
Toxemias of pregnancy
Following administration of
a) Chloroform
b) Arsphenamine

It has recently been said that this test should be used always in connection with antiluetic therapy as it is even more important than the Wassermann.

- 2 The detection of passive congestion of the liver due to myocardial failure in

{ Heart disease
 { Emphysema
 { Arteriosclerosis
- 3 In differentiation of primary and secondary anemias (Normal values are the rule in nonhemolytic anemias)
- 4 Involvement of liver and biliary passages in the presence of a carcinoma
- 5 The differential diagnosis of cases of abdominal colic Following biliary colic the serum bilirubin is elevated, although in about 60 per cent of cases not to a degree or for a length of time sufficient to color the sclera or skin Here the detection of a latent jaundice is of great value

The icterus index is therefore of considerable practical value It is of value in giving quantitative information as to the amount of biliary dysfunction and is at present the best single functional test of the liver available It can be done easily and readily in the office It is also of value in the differentiation of the primary from the secondary anemias

SUMMARY

A simple method of combining the determination of the sedimentation rate of the erythrocytes, the corpuscle volume, and the icterus index has been presented These tests, when done in this manner, give a great deal of worthwhile information which we should dislike very much to do without in our studies of the chronic ambulatory invalid

A RUBBER MASK FOR DETERMINATION OF OXYGEN CONSUMPTION OF THE DOG*

BY ALFRED BLALOCK, M D, NASHVILLE, TENN

IN STUDIES upon the cardiac output of the dog, a great deal of difficulty was encountered in obtaining accurate determinations of the oxygen consumption due to inability to effect an air-tight connection between the animal's mouth and the spirometer Many methods were tried in attempts to overcome this difficulty, but none were entirely satisfactory The most successful method consisted of the use of a mask which was made of plaster of Paris, but it was necessary that each animal have an individual one which was shaped according to the contour of the face The mask was then covered with paraffin, and plastacene was employed to make a good connection between the animal and the spirometer In addition to the fact that leaks were

*From the Department of Surgery of the Vanderbilt University
 Received for publication September 6 1926

encountered rather frequently, it was unsatisfactory because it required the constant attention of one person in order to hold it in place

Following the suggestion of Dr Beverly Douglas, a specially constructed mask was ordered from The Baumann Rubber Company of New Haven Conn. It is made of rather easily pliable rubber and has the shape of a funnel. The diameter of the smaller end is such as to permit of an air tight connection with a Benedict spirometer. The larger opening is of sufficient size to allow its being placed over the animal's mouth without undue stretching. This, in turn, is surrounded by a separate hollow rubber tube which can be inflated through a smaller rubber tube leading off from the side. Before applying

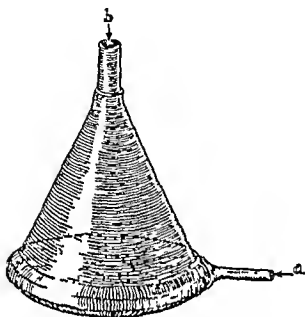


Fig 1—Lateral view of mask

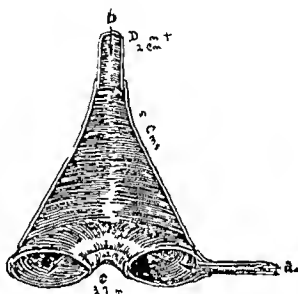


Fig 2—Hemisection view of mask

the mask, the corners of the animal's mouth are anchored in place by several turns of a circular rubber dam. The mask is then placed over the rubber dam, the circular tube is inflated tightly, and an air tight connection secured. It is very important that the circular rubber dam should not be applied too closely to the tip of the animal's nose, as this would result in respiratory obstruction.

This method has been used in at least two hundred determinations of the oxygen consumption of dogs varying in weight from five to eighteen kilograms and no difficulty has been encountered. In addition to the accuracy which it insures, it usually allows the observer to continue with the experiment, since it is not necessary to hold the mask in place. The amount of dead space is almost negligible.

THE GLUCOSE TOLERANCE TEST*

BY W B LEWIS, M D, BATTLE CREEK, MICH †

THE glucose tolerance test is daily coming to have a greater significance. It is of particular value in those early borderline conditions where diagnosis is not clear-cut and easy. Richter¹ has well said in regard to gallstone disease that "it is not gallstone disease at all on which we should focus our attention, but the antecedent condition of which gallstone disease is only a late complication." And likewise, in disturbances of carbohydrate metabolism it is not the well-developed condition of diabetes that we should be most interested in, but those early changes before the damage becomes great.

Several methods for detecting the prediabetic state have been proposed. In 1914, Peter Bergell² of Berlin, stated that the urine of a prediabetic free of sugar had a greater solvent power for cupric hydroxide than normal urine. We have used this test extensively. A report of this work will appear later. Concerning some cases, it gave early and valuable information. In many others it did not.

In 1918, S R Benedict³ proposed his sodium picrate method for normal sugar in the urine, and on the basis of results with this method he drew the conclusion, "If the total sugar elimination amounts to more than one and a half grams per day, the diet should be altered until this figure is reached as the upper limit."

In 1922, Folin and Berglund⁴ adapted the Folin and Wu⁵ blood sugar method to the determination of the normal sugar in the urine and published their classical research on carbohydrate metabolism. Their normal sugar method was not proposed as a prediabetic test, but rather the contrary, it showed that *the reducing substances of normal urine are mainly not dextrose but*, "First, foreign, unusable, carbohydrate materials present in grains, vegetables and fruits, and second, decomposition products due to cooking, canning and baking of such food. The sugar of normal urine consists, therefore, of a motley variety of carbohydrate products and carbohydrate derivatives, including di- and polysaccharides." Therefore, the elimination of one or two grams of such substances per twenty-four hours is not an indication of the prediabetic state but dependent almost entirely on the diet.

It is the use of this method of Folin and Berglund for the study of the urine in conjunction with blood-sugar curves following the ingestion of dextrose that we believe gives one the most reliable information regarding early disturbances of carbohydrate metabolism.

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.

†From the Clinical Laboratories of the Battle Creek Sanitarium.

METHOD

- 1 At 7 00 A M, patient empties the bladder and drinks one glass of water
- 2 At 8 00 A M, one hour specimens of urine and fasting blood sugar are obtained Patient is then given 100 gm of pure dextrose dissolved in water with the juice of one lemon and made up to 200 cc, also one glass of water
- 3 At 8 30 A M, blood and urine specimens are obtained
- 4 At 9 00, 10 00, 11 00 and 12 00 o'clock, blood and urine specimens are collected

Blood sugar is determined by the Folin and Wu method Each urine specimen is tested for sugar first qualitatively with the Folin McIlroy^a reagent Then, on the basis of the amount of reduction obtained, the urine is diluted and the sugar determined quantitatively by the Folin Berglund normal sugar method By the use of tables the dilutions and calculations are relatively simple Urinary sugar is expressed as milligrams per hour

RESULTS

TABLE I

TYPICAL RESULTS SELECTED FROM 600 CASES

NAME		FASTING	1/2 HOUR	1 HOUR	2 HOUR	3 HOUR	4 HOUR	
Mr M H	Blood Sugar	83	172	1	96	78	83	Normal
	Urinary Sugar	5	0	0	18	15	18	
Mr L S	Blood Sugar	80	113	133	83	74	83	"
	Urinary Sugar	10	7	13	23	20	15	
Mr D H	Blood Sugar	83	106	84	88	81	66	"
	Urinary Sugar	10	7	0	14	12	9	
Mrs G E	Blood Sugar	76	05	101	91	80	82	"
	Urinary Sugar	28	5	9	21	10	9	
Miss R A	Blood Sugar	91	105	95	70	65	87	"
	Urinary Sugar	21	13	8	10	1	10	
Dr W T M	Blood Sugar	111	167	—	152	140	—	Slight disturbance
	Urinary Sugar	0	89	—	99	37	—	
Mr P R	Blood Sugar	81	167	91	70	7	01	"
	Urinary Sugar	13	13	60	22	15	14	
Mr L A	Blood Sugar	100	194	208	201	114	05	"
	Urinary Sugar	5	37	1072	2240	1114	1318	
four months later	Blood Sugar	77	159	199	159	100	7	"
	Urinary Sugar	8	13	60	201	35	13	
Mrs L G	Blood Sugar	106	191	157	240	260	103	"
	Urinary Sugar	26	13	119	073	79	118	
Mr E C M	Blood Sugar	130	240	310	273	162	130	Moderate disturbance
	Urinary Sugar	13	121	1753	141	1177	160	
Mrs G F	Blood Sugar	133	217	345	370	102	128	"
	Urinary Sugar	14	1	10	141	1114	110	
Mr W B C	Blood Sugar	154	290	361	313	20	17	"
	Urinary Sugar	29	210	202	620	1317	910	
Mrs K H	Blood Sugar	144	313	364	31	278	119	"
	Urinary Sugar	07	77	3664	8765	7413	234	
Mrs A B	Blood Sugar	182	286	333	09	308	222	"
	Urinary Sugar	18	11	220	1064	2400	80	
Miss M S	Blood Sugar	182	333	400	300	286	211	Severe disturbance
	Urinary Sugar	14	33	6400	9125	5265	92	
Mr M D	Blood Sugar	200	357	435	16	351	270	"
	Urinary Sugar	—	735	100	13000	11711	6912	
Mr H S	Blood Sugar	213	203	304	117	177	280	"
	Urinary Sugar	13	11	123	1615	8000	152	
Mr S H	Blood Sugar	215	364	370	377	209	211	"
	Urinary Sugar	1800	3000	5083	10150	8900	1590	

Fig 1 Normal These curves are typical of over 100 obtained from students and others Fasting blood sugar of 80 to 100, rising to a maximum at one-half hour, usually less than 150, then rapidly falling, usually below the starting point at the second or third hour and then rising to near the starting point

The urinary sugar when expressed in mg per hour forms a curve that corresponds closely with the blood-sugar curve In this normal it is 10, 9, 10, 16, 18, and 15 mg per hour, relatively very constant *It is thus close paral-*

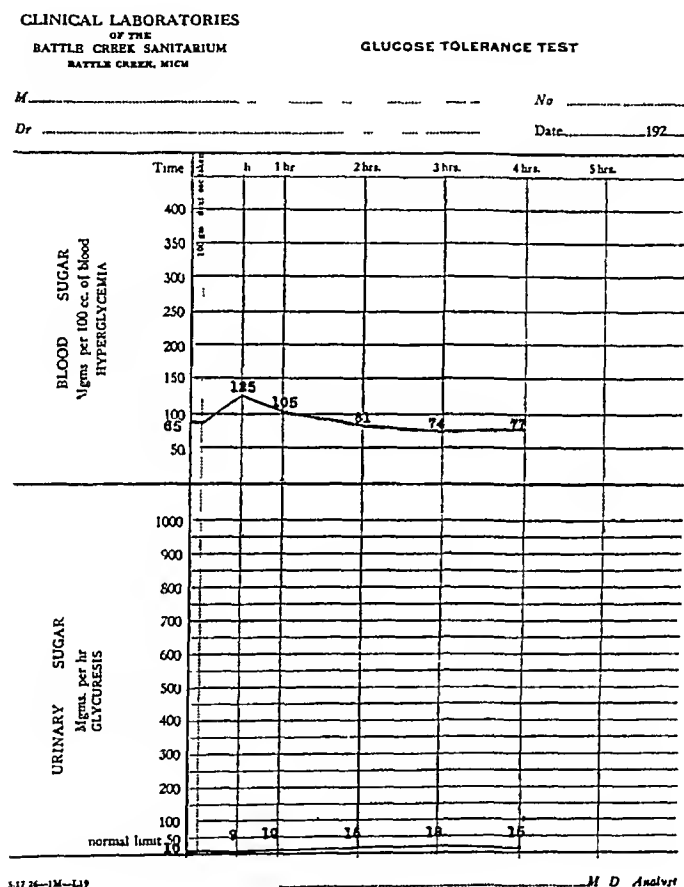


Fig 1

leling of the urinary sugar curve with the blood-sugar curve that I wish to call special attention to

Fig 2 Slight disturbance In this case the patient had had a long siege of boils for over a year without making any progress in getting rid of them The fasting blood sugar is 111 and rises to a maximum of 182 at the second hour and at the third is still above the starting point The urinary sugar closely follows this, starting at 9 mg per hour it rises to 372 at the third hour Following the test he markedly lowered the carbohydrates in his diet, and in three weeks the boils had disappeared and have not returned in nearly a year

Fig 3 Moderately severe disturbance This is an interesting case, showing the diagnostic value of the test A fasting blood sugar of only 135 and no increase of sugar in the urine Her main complaint was that of easily tiring and weakness Had no suspicion of diabetes At one hour the blood sugar reached a maximum of 333 and at three hours was still 174 The urinary sugar curve is very similar, showing at the second hour a maximum of 66 gm

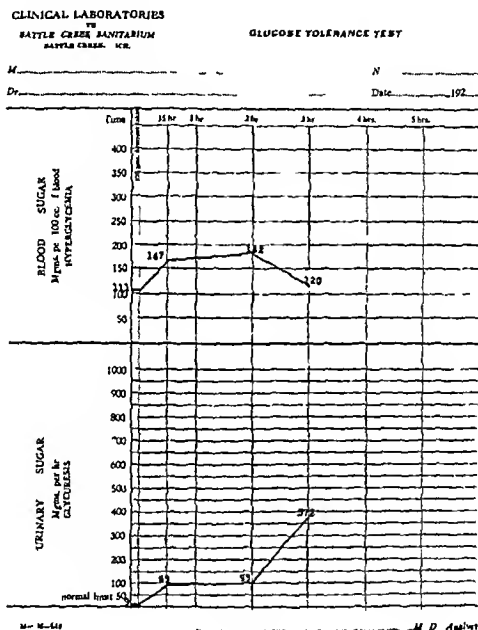


Fig 2

Fig 4 Severe disturbance This patient also had not been diagnosed as a diabetic, although the fasting blood sugar was 213 There was a high renal threshold as there was no increase above normal of the urinary sugar A typical diabetic blood sugar curve which at the end of four hours is still 286, with large amounts of sugar in the urine

DISCUSSION

1 Our results confirm Fohn's in showing that there is a very definite renal threshold for dextrose

Until the renal threshold is passed, the amount of reducing substances in the urine is relatively very constant

The average healthy individual does not exceed this threshold and there is, therefore, no increase of normal sugar in the urine above 40 or 50 mg per hour as a maximum after taking 100 gm of dextrose. We, therefore, have adopted this figure as our maximum normal limit.

2 Practically all of our cases showing a fasting blood sugar of 130 or more, by the tolerance test, showed some degree of disturbance of carbohydrate metabolism. Therefore, a fasting blood sugar of 130 should be looked upon with grave suspicion.

3 But, what is even more important from the diagnostic standpoint is

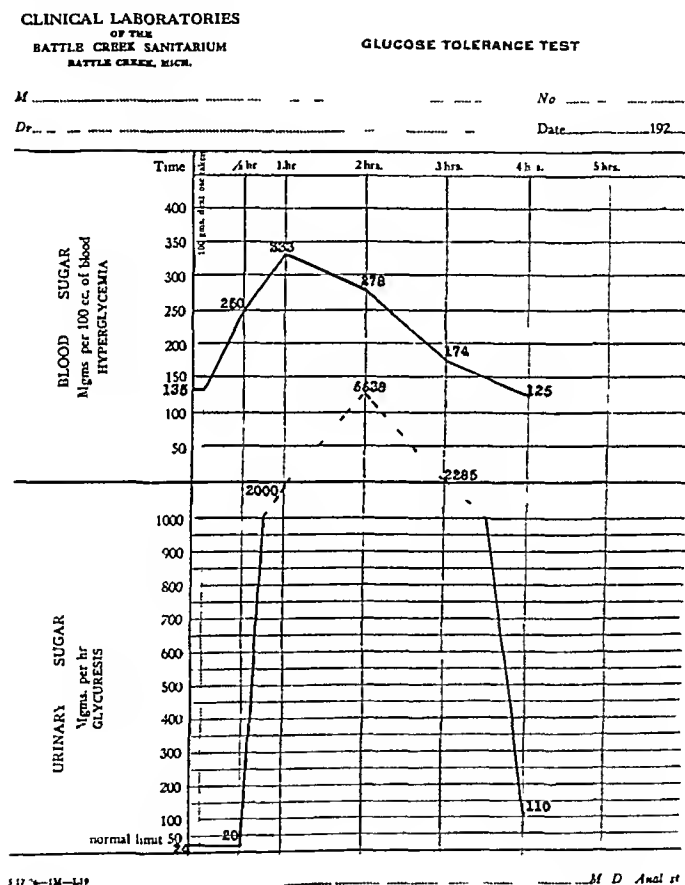


Fig 3

the large number below 130 blood sugar that show disturbances of carbohydrate metabolism of varying degree, some quite severe. Approximately 32 per cent of our series of 600 on *sick people*.

A fasting blood sugar within the usually accepted normal limits is therefore no guarantee that disturbances of carbohydrate metabolism are not present when the organism is subjected to a slight strain, as in the tolerance test.

4 Because of the need of more frequent testing of carbohydrate metabolism, I would suggest, based on the results in this series, the following as

a preliminary test that may be carried out by any physician. Give the patient 100 gm of dextrose with instructions to save the urine for one hour previous to taking it, in the morning, without breakfast, and for four hours following, in hourly periods. Have the patient bring these specimens to the physician's office where he will run a qualitative sugar test on each, and from this he will get a very good idea whether any abnormality is present. If there is, then have a laboratory equipped for blood chemistry do a complete tolerance test.

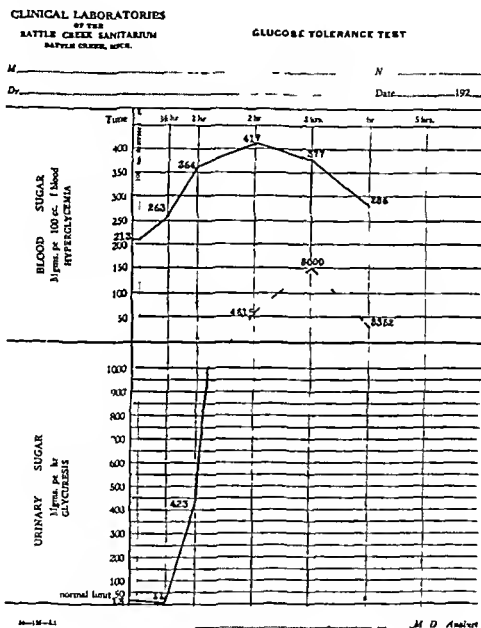


Fig. 4

5 In this series the test was found of value in the following conditions

- 1 Diabetes mellitus
- 2 Renal diabetes
- 3 Hyperthyroidism
- 4 Furunculosis
- 5 Pruritus
- 6 Testing the susceptibility of near relatives of diabetics
- 7 Pituitary disturbances
- 8 Hypoadrenalism
- 9 Arthritis

OXYGEN THERAPY A METHOD OF ADMINISTRATION AND APPARATUS

BY PAUL ROTH, M.D., BATTLE CREEK, MICH

OXYGEN therapy is indicated primarily for the relief of anoxemia and various disturbances to which it leads. The seriousness of the effects of oxygen want has been appreciated only in recent years and the clinician in general still needs to be awakened to the advantages and effectiveness of the more modern and improved methods of oxygen administration.

The surprisingly slow progress made in the therapeutic uses of oxygen from which so much has been justly expected ever since that element was discovered, is due, as well stated by Haldane¹ to (1) "Failure to understand both the immediate and the remote effects of oxygen want" (2) "Failure to appreciate that the longer the period of want of oxygen lasts, the greater is the progressive damage done to the central nervous system, heart, and other organs and the slower and more difficult does recovery become. A deficient oxygen supply to the body, if allowed to continue, is undoubtedly a matter of very serious moment to a patient, and should be prevented, if this is at all possible." Haldane further says "Anoxemia is not only always dangerous, but its injurious effects may soon become irreparable though the anoxemia may have been effectively corrected."

When this was written, seven years ago, this author remarked "It seems evident that there is a wide field for the therapeutic use of oxygen, and probably oxygen will before long become one of the commonest remedies." Today oxygen is, as yet, anything but a common remedy in spite of its having been for years a very common article obtainable at a relatively low cost.

It is hoped that every clinician now knows that the feeding of oxygen in whiffs from a rubber bag or from a tank, is obsolete and, with but rare exceptions, useless. In most cases it should be administered by the hour or half hour at least, more or less frequently repeated, and often as continuously as possible.

The possibility of obtaining in certain cases definite results from even shorter periods of administration must not be disregarded. For instance, H. Simon² reports that in six of ten cases with nephritic and arteriosclerotic hypertension, a permanent lowering of the blood pressure was obtained as the result of inhaling for six to eight minutes, several times a day usually, about six liters of oxygen per minute.

The ideal method of oxygen therapy is by means of a specially built room, the atmosphere of which is mechanically regulated to contain from 30

*Abstracted from paper read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.
From the Research and Metabolism Laboratories of the Battle Creek Sanitarium.

to 60 per cent of oxygen with no excessive amount of CO or moisture and maintained at a desirable temperature at all seasons of the year. Such facilities are necessarily elaborate, costly to install and to operate. Fortunately excellent results are obtainable by less elaborate equipments.

The simple methods of oxygen feeding, by means of a mask, mouth piece, or nasal tube while suitable for short periods as in emergency work, have generally been found to seriously interfere with the comfort of the patient. Nevertheless such means will never be entirely ignored in the absence of better ones. Attention should be called to the fact that there are now scattered all over the land a large number of respiration apparatuses for the estimation of the basal metabolic rate. Without any modification they are very suitable oxygen feeders though they require much attention when used for this purpose.

The comfort of the patient must be considered as well as the efficiency of the method of administration, therefore any contrivance which interferes with the movements of the patient especially of the head are objectionable because they invariably become intolerable in prolonged use.

The bed tent described by Hill and also by Barach and Binger has been reported to be very practical and efficient. The chief disadvantage of the full bed tent is that it does not allow free access to the patient to tender usual nursing care and treatment without suspending the oxygen feeding. It is also more cumbersome and wasteful of the oxygen required to maintain an adequate concentration.

The hood or head tent proposed by the writer and previously described² has continued to give entire satisfaction. The skirt of this hood is made fuller at the lower part making it possible to enclose the head, shoulders and arms if desired. Fig. 2.

Analyses have shown that with a subject weighing 70 kg. a flow of oxygen of 3, 4, 5, 6 or 7 liters per minute will maintain in the hood approximately and respectively 30, 40, 50, 60, or 70 per cent of oxygen. Immediately after the hood is properly adjusted, oxygen is freely admitted in the apparatus for about one minute. The flow is then reduced to that necessary to maintain from 40 to 50 per cent concentration of oxygen in the air inhaled by the patient. Barach has confirmed the fact that concentrations of oxygen exceeding 70 per cent become harmful when breathed for a long period of time.³

The administration of oxygen in a hood or tent enclosing the head only or also the shoulders and arms, presents the following distinct advantages.

The comfort of the patient is not interfered with because the movements of the body or of the head are not restricted by mouth or nasal connections or the use of a mask.

The administration of oxygen at any desirable concentration is easily secured and uniformly maintained chiefly because it is relatively easier to keep well ventilated a small hood than a tent covering the entire bed or a larger enclosure of any kind.

The patient is readily accessible at all times for general care and treatment, which can be administered without interfering with the oxygen feeding.

The air and oxygen supply is readily maintained pure and sweet. The fouling by gases from the bowels, or by other noxious emanations from the body surface, increased often by the application of compresses, fomentations, packs, diathermy or of dressings and ointments, is avoided.

The hood can also be more easily kept comfortably cool, thereby securing the distinct therapeutic advantages of cold air, which is a valuable respiratory and cardiac tonic. At the same time the patient can be cared for in a properly heated room, thus avoiding the dangers of exposure to cold.

This method of administration has proved to be efficient while the cost of operation is minimized. Such a simple and transportable equipment as the one here described can readily be installed at the residence of the patient. The

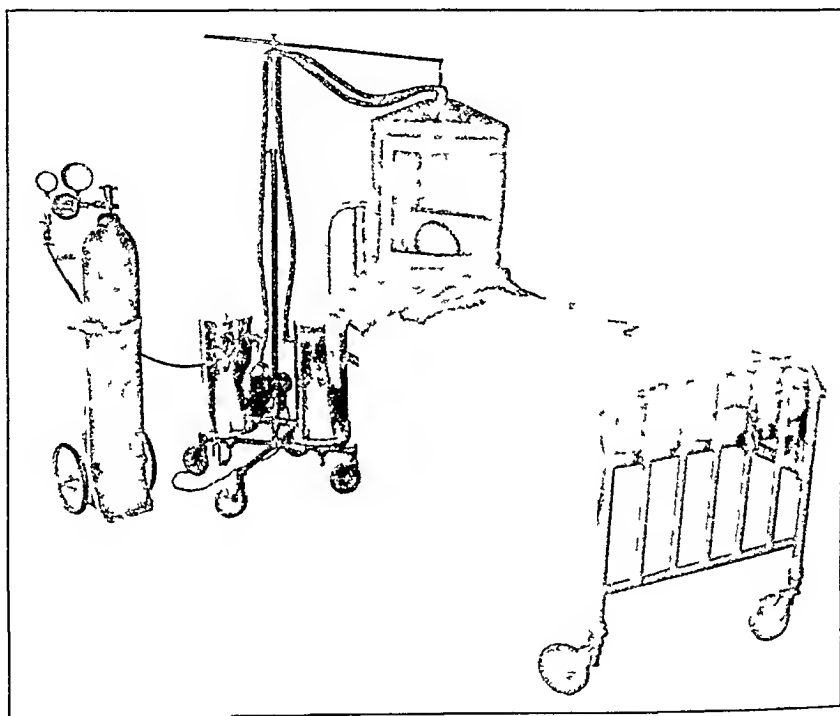


Fig 1

demand for such facilities already exists in practically every hospital and medical institution.

The introduction and operation of an equipment for oxygen therapy demands intelligent supervision. For plausible reasons, the clinical pathologist will probably be more often consulted than anyone else and his expert cooperation enlisted for this purpose.

It is safe to predict that oxygen therapy, properly conducted in its legitimate field of usefulness, will save more lives than resuscitation apparatus have or ever will in emergency work.

This oxygen therapy outfit (Fig 1) is a reconstruction of the apparatus previously described.³ The purpose of the builder* was to dispense with the

*Warren E Collins 555 Huntington Ave Boston Mass

use of the cabinet and group the various parts into a more compact and more easily transported unit. He also devised for this apparatus a remarkably efficient and quiet running ventilator operated by a universal motor.

To the left of this ventilator is the air cooler which is filled with pieces of ice the size of one or two fists, over which the air passes directly instead of through metal coils. This method of air refrigeration was suggested by Dr. Alvau L. Barach.⁴ A U shaped trap drains the cooler. The other large can on the right contains the soda lime for CO₂ absorption.

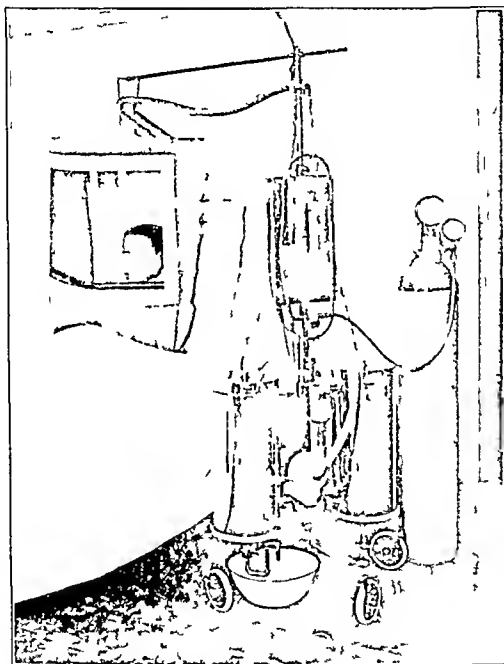


FIG. 2

The flow of oxygen is measured by means of a special Oxnoid reducing valve which indicates on the smaller dial the flow in liters per minute. The wash bottle type of flow meter previously described³ is also shown in Fig. 2. This will answer the purpose with the use of any ordinary reducing valve.

Barach also advised to admit the fresh air in the hood through the perforated tube formerly used to remove the expired air. The fresh cool air is thus blown directly toward the face. While this presents an advantageous feature, the writer believes that the ideal position for the perforated de-

livery tube is, as shown in Fig 1, above and behind the head of the patient. It is there out of the way, does not obstruct the view through the large single celluloid window and facilitates the handling of the hood preparatory to or during treatment. Besides, if the fresh air is introduced from the rear it will not meet the intermittent current of expired air coming from an opposite direction, mixing with it more or less and reducing its oxygen concentration before it can be inhaled. The expired air will thus be more readily carried away from the vicinity of the nose or mouth and, being warm, will tend to rise toward its outlet at the top of the hood.

Fig 2 shows above, mounted on a board, the very simple apparatus described by Binger⁵ for the rapid determination of oxygen. With it the concentration of oxygen in the inspired air in the hood can readily be ascertained as frequently as necessary.

, SUMMARY

1 Deficiency of oxygen supply in the living tissues soon results in grave disturbances, which often are more easily prevented than relieved.

2 Oxygen should become one of the commonest remedies.

3 The advantages of oxygen administration in a small tent or hood, enclosing the head only, are discussed.

4 This method permits the administration of oxygen in combination with the therapeutic use of cold air in a properly heated room.

5 The apparatus can quickly be installed in any home as well as at the hospital and operated from an ordinary electric light socket.

6 Several additional improvements in the apparatus are presented.

REFERENCES

- ¹Haldane, J. S. Recent Developments in the Therapeutical Use of Oxygen in Contributions to Medical and Biological Research, 1919, Paul Hoeber, New York, 1, 549.
- ²Simon, H. The Action of Oxygen on Blood Pressure, *Klin. Wchnschr.*, October 1, 1925, iv, 1897-1944.
- ³Roth, Paul. Improved Apparatus for the Therapeutical Administration of Oxygen, *The Modern Hospital*, xxi, April, 1924, 404, 405. *Bulletin of the Battle Creek Sanitarium and Hospital Clinic*, July, 1924, vii, 3.
- ⁴Barach, A. L. The Effect of the Inhalation of Oxygen Over Long Periods of Time in Normal Rabbits and in Rabbits with Pulmonary Tuberculosis, *Am. Rev. Tuberc.*, April, 1926, xii, 293.
- ⁵Binger, C. A. L. The Construction and Management of an Oxygen Chamber, *Mod. Hosp.*, Feb., 1925, xiv, 186.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A KILDUFFE MD ABSTRACT EDITOR

Kendall A. I and Keith H. B. Nature of the Soluble Proteolytic Enzyme of *B. Proteus* Jour Infect Dis, March 3 1926, xxxviii No 3, 193

A report of a quantitative study of the changes induced in gelatin by the soluble enzyme of *B. proteus*. The proteolytic enzyme of this organism produces a rapid change in the gelatin molecule of the tryptic rather than peptic type, the rate and extent being equal or even greater than that of commercial trypsin under parallel conditions

Study of the flora of nurslings (Kendall A. I, Day, A. A. and Walker A. W. Chemistry of the Intestinal Flora of Nurslings Jour Infect Dis, March 1926 xxxviii No 3, 200), indicate that the nitrogenous changes induced by bacteria are slight. Proteolysis of bacterial origin is minimal. The striking change is a pronounced utilization of glucose and lactose, principally the latter

In artificially fed infants (Kendall A. I, Day A. A. and Walker, A. W. Chemistry of the Intestinal Bacteria of Artificially Fed Infants Jour Infect Dis. March, 1926, xxxviii, No 3, 205), the feces induce putrefactive changes in sugar free mediums and fermentative changes when carbohydrates are added

In adults (Kendall, A. I, Day A. A. and Walker A. W. Chemistry of the Intestinal Flora of Normal Adults Jour Biol Chem, March, 1926 xxxviii, No 3, 211), the chemical picture is one of unexpected uniformity in the nitrogenous changes

Moderate proteolysis occurs in mediums containing no utilizable carbohydrate which is nearly entirely suppressed when carbohydrates are added

When the intestinal flora contains *B. aerogenes capsulatus* there is marked proteolysis

When abnormal numbers of gas bacilli are present (Kendall, A. I, Day, A. A., and Walker A. W. Chemistry of the Intestinal Flora of Man Containing Abnormal Numbers of Gas Bacilli Jour Infect Dis, March, 1926, xxxviii No 3, 217), the outstanding feature is marked proteolysis accompanied by extensive deamination resulting in the accumulation of ammonia in the culture

In carbohydrate-containing mediums these activities are almost entirely suppressed

The influence of insulin upon the utilization of glucose in the diabetic suggested the study of its possible effect on non-glucose fermenting bacteria (Kendall, A. I. Non-glucose Fermenting Bacteria and Insulin Jour Infect Dis, October, 1925, xxxvii, No 4, 329) with entirely negative results

The results of the adding insulin to milk glucose insulin cultures of *B. bulgaricus* and *B. acidophilus*, as shown by detectable increase in titratable acidity were indefinite and remain an open question (Kendall, A. I and Ishikawa M. Effect of Insulin on Cultures of *B. Bulgaricus* and *B. Acidophilus*. Jour Infect. Dis, October, 1925 xxxvii, No 4 333)

Peters J. B. Bulger H. A. Eisenman A. J. and Lee C. Concentration of Acids and Base in Normal Plasma. Jour Biol Chem, January 1926 lxxvii, No 1 141

With the primary purpose of obtaining an insight into the mechanism of the changes in the blood and tissue hydration in nephritis the authors report an intensive study of three years duration

In all cases chloride and bicarbonate, and in most cases the proteins of the plasma were determined. Additional studies were added as they seemed indicated

This, the first of a series of papers, describes in detail and at length the experimental methods and technique employed and these cannot well be abstracted without almost a transcription of the entire paper

From a study of normal individuals the following conclusions were formulated

The total base, inorganic acids, bicarbonate, chloride, phosphate, and protein were determined

The difference between total base and the sum of the base combining powers of the acids enumerated gives a measure of the organic acid and sulphate. The latter is present in negligible amounts only so the "undetermined" acid must be practically equivalent to organic acid

Normal serum contains 147 to 161 millimols of monovalent base, 138 to 148 millimols of this base being combined with the four acids, protein, bicarbonate, chloride, and phosphate. For hospital patients these limits should probably be extended to 145 to 167 for total base and 135 to 155 for total acid. The organic acid never exceeded 20 millimols in normal persons

There is a general tendency for protein, bicarbonate, and chloride to reciprocate in their changes in the maintenance of a constant level of acid and total base

When the CO_2 tension is increased from 30 to 60 mm at 38°C (II, Eisenman, A. J., Bulger, H. A., and Peters, J. P. The Effect of CO_2 Tension on the Concentration of the Acids of the Plasma of Oxygenated Blood. *Jour Biol Chem*, January, 1926, lxxvii, No 1, 159), the sum of the base combining powers of the acids HCO_3 , plus Cl plus protein of the plasma of oxygenated blood increases about 2 millimols. In this change HCO_3 increases 5 millimols, the extent being determined chiefly by the hemoglobin concentration or volume of the blood cells. The average change of plasma volume amounts to -0.6 volumes per cent while the base combining power of the proteins diminishes about 0.8 mm

Cl decreases by about 2 millimols. Because base does not traverse the cell membrane the loss of water from plasma to cells results in a concentration of base that neutralizes the excess acid

The general conception that arterial and venous blood differ as regards electrolyte equilibria only in so far as they contain more or less carbon dioxide and oxygen is erroneous (Peters, J. P., Bulger, H. A., and Eisenman, A. J. III, The Differences Between Arterial and Venous Blood. *Jour Biol Chem*, January, 1926, lxxvii, No 1, 165)

Arterial and venous blood may contain also different amounts of water and chloride and the carbon dioxide absorption curves may also differ. The changes that occur while the blood is traversing the tissues affect the different components to different degrees and in different directions for reasons not yet determined

The end result on the plasma acids is an average alteration of 2.5 millimols, usually an increase. The maximum variations encountered were +5 and -2.5 millimols

Venous obstruction leads to a transfer of water from the blood to the tissues and a concentration of the proteins (Peters, J. P., Bulger, H. A., Eisenman, A. J., and Lee, C. IV, The Effect of Stasis, Exercise, Hyperpnea, and Anoxemia, and the Causes of Tetany. *Jour Biol Chem*, January, 1926, lxxvii, No 1, 175). In brief, vigorous exercise, considerable lactic acid and an excess of carbonic acid are formed and the serum P_H falls. Chloride remains unchanged. Bicarbonate cedes some base to the organic acid, but the major portion of the latter is neutralized by base yielded from the tissues

If over ventilation is produced rapidly symptoms of tetany appear when the P_H has risen not more than 0.2. Although the total CO_2 falls, the CO_2 capacity is unaltered

Organic acid is considerably increased. The base required for neutralization of foreign acids is largely derived from the chlorides, which are diminished

The reaction of electrolytes to oxygen want varies according to the respiratory response. Any given disturbance of electrolyte equilibrium evokes a train of reactions in all the other electrolytes tending to restore equilibrium

Studies in various pathologic conditions are reported (Peters, J. P., Bulger, H. A., Eisenman, A. J., and Lee, C. V, Miscellaneous Pathologic Conditions. *Jour Biol Chem*, January, 1926, lxxvii, No 1, 219)

The effects of vomiting are highly variable and probably depend on the nature of the vomitus, the severity and duration of the emesis, and the degree of inanition produced. The most frequent result is a reduction of chloride with or without a reduction of base. The bicarbonate level is irregular

Vomiting of HCl is not essential for this picture as it was encountered with esophageal obstruction in the absence of vomiting

In a series of infections bicarbonate was generally about normal. In pneumonia it is usually normal while chloride is almost invariably low

Anemia and polycythemia have no characteristic influence on the acids or base of the serum.

In diabetes (Peters, J. P., Bulger, H. A., Eisenman, A. J., and Lee, C. VI, *Studies of Diabetes Jour. Clin. Invest.*, December, 1925, 11, No. 2, 167) ketosis of considerable severity may develop without appreciably affecting the bicarbonate of the plasma. In these cases chloride is usually reduced and the base required for the neutralization of the organic acid is evidently derived from the chloride

In severe diabetic acidosis the base required for neutralization of ketone acids is ceded by both bicarbonate and chloride. Chloride reduction may occur very rapidly and without augmented chloride excretion, indicating that the chloride ion is merely transferred to the tissues. In profound diabetic toxemia the salt content of the blood and probably of the tissues is seriously depleted. The bearing of these phenomena on the treatment of diabetic toxemia, ketosis, and acidosis is discussed in this paper

In nephritis (Bulger, H. A., Peters, J. P., Eisenman, A. J., and Lee, C. VII, *Factors Causing Acidosis in Chronic Nephritis Jour. Clin. Invest.*, February, 1926, 11, No. 3, 413), it appears that a reduction of total base and an increase of undetermined acids are the most significant factors causing acidosis in chronic interstitial and arteriosclerotic nephritis phosphates being less important. The degree of acidosis seems greatly influenced by variations of plasma chloride. With high chloride bicarbonate may be extremely low

Beeson B. B. and Church J. G. Superficial Yeast Infections of the Skin and of Its Appendages. *Arch. Dermat. and Syph.* May, 1926, 111, 644

The authors review the literature of yeast infections of the skin, call attention to the increasing number of cases reported, and report their study of a case of interdigital infection.

Cases with a geographic, well marked border along with a loosened fringe of epidermis should be studied with yeasts in mind

Many cases called "intertrigo" may be due to yeasts. Yeast infections seem capable of producing a variety of skin lesions and in lesions identical from a clinical standpoint different yeasts may be found.

McLean, A. B. and Sullivan E. C. Blood Sugar in Status Thymicolymphaticus. A New Theory as to the Cause of Sudden Death. *Am. Jour. Med. Sc.*, May, 1926, clxxi, No. 5, 659

The clinical picture in cases of status death being somewhat similar to that seen in "insulin shock," blood sugar determinations were made and a marked hypoglycemia found. In three cases the blood sugar values were 42, 52 and 57 mg per 100 c.c. In one case of suprarenal hemorrhage 25 mg were found. In six cases of convulsions produced by conditions other than status thymicolymphaticus, and in six cases where determinations were made within one half hour of death, normal values were found.

Acute suprarenal insufficiency is suggested as the immediate cause of sudden death in status thymicolymphaticus

Hager B. H. and McGath T. B. Etiology of Incrusted Cystitis with Alkaline Cystitis. *Jour. Am. Med. Assn.*, Oct. 31, 1925, lxxxv, 1352

Cultures from the urine in this condition gave a constant growth of a bacillus having the general characteristics of the bacillus found in the nasal discharges from ozena (*Salmonella fetida*), but which possesses sufficient cultural differences to cause the authors to regard it as a distinct species to which they give the name *Salmonella ammoniae*. The organism is capable of breaking urea into ammonia and carbon dioxide in a very short time.

The organism was found consistently in seven cases and when injected into the bladder

ders of guinea pigs in whom a chemical cystitis had been induced, an alkaline incrustation was produced

Hager, in a later paper (Hager, B H A Contribution to the Etiology of Calcareous Pyelonephritis Jour Urol, February, 1926, xv, No 2, 133) demonstrated that this organism can invade the kidney pelvis and, under favorable conditions, produce a calcareous pyelonephritis

Greenwald, H M, and Eliasberg, H The Pathogenesis of Death from Burns Am Jour Med Sc, May, 1926, clxxi, No 5, 682

In two cases which presented a marked hypoglycemia experiments on rabbits (10) demonstrated that the cause of death in these animals may be divided into two stages a. Initial stage due to shock and accompanied by hyperglycemia due to hyperactivity of the suprarenals, b Secondary stage, due to degenerative changes, particularly in the suprarenals The administration of adrenalin is indicated only in the secondary stage of suprarenal exhaustion

McQuarrie, I, and Shohl, A T A Colorimetric Method for the Determination of the P_{H} of Cerebrospinal Fluid. Jour Biol Chem, December, 1925, lxxvi, 2

The authors devised an apparatus (made by the Empire Laboratory Supply Co, New York), on the principle of the Van Slyke apparatus whereby loss of CO_2 , contact with air, and transfer are all avoided

The apparatus is figured in the paper

Method—Measure into the apparatus as many tenths of cc of Hastings' indicator solution (0.0075 per cent phenol red) as of cc of spinal fluid which it is expected to use Connect the upper capillary tube directly to the lumbar puncture needle by means of a rubber tube bearing a glass Luer adapter previously sterilized, observing sterile precautions the while Allow the fluid to escape under its own pressure through the side of the three-way stopcock which has been turned so as not to be connected with the apparatus When all air has been removed from the capillary tube, allow the spinal fluid to enter the apparatus by turning the stopcock and holding the bulb in such a position that its mercury level is at or very slightly below that in the sampling tube The flow of spinal fluid is stopped when the desired amount has entered Place a pinchcock at the lower end of the apparatus Immerse in a water bath at 38° for five minutes and compare with the bicolorimetric standards

A comparison of the blood and spinal fluid shows that the two normally have the same P_{H} 7.35 to 7.40 ± 0.02

Shivers, C H deT Clinical Value of Bismuth in Treatment of Syphilis Arch Dermat and Syph, October, 1924, v, 414

The author concludes that

1 Bismuth should not be substituted for arsenic in primary or secondary syphilis, except in patients resistant to arsenical treatment

2 Bismuth is effective, clinically, in the treatment of all forms of tertiary syphilis.

3 In the treatment of neurosyphilis, bismuth in some cases has proved itself superior to arsenic.

4 Bismuth should be tried in all patients who do not tolerate the arsenicals.

5 Bismuth should be given with caution in all patients with faulty elimination

6 The absorption of this drug should be carefully studied, and if possible, the number of treatments given should depend on the roentgenologic findings

He reports three cases in which chills, headache, bleeding gums, and a sense of oppression in the chest were noted due to a cumulative toxic effect

Taccone, G A Cerebrospinal Fluid Test Pediatrics, February, 1926, xxvii, 131

A five per cent solution of bichromate of potash is heated and filtered, and after cooling, a number of drops of trichloroacetic acid equivalent to the number of cubic centimeters of the solution are added The reagent thus prepared will keep indefinitely Addition of

trichloroacetic acid may also be made as occasion requires. For example from three to four drops of the acid may be added at the time to the three to four cc of the solution of bichromate of potash which are necessary for each test. Into a test tube of one centimeter in diameter which contains from three to four cc of the reagent is introduced one half of one cc of the cerebrospinal fluid which is intended for examination and which has previously been freed from blood. The cerebrospinal fluid should be added drop by drop to the walls of the tube by means of a fine pipette during gradual application of heat in such manner that the two fluids are superimposed but are not mixed.

A positive reaction is said to manifest itself primarily by the formation in the zone of contact of the two fluids of a ring which in the cerebrospinal fluid of sufferers from meningitis becomes immediately denser and thicker than that produced by normal or by meningo-encephalic types of cerebrospinal fluid, in which the quantity of albumin is either normal or is only slightly increased (since density and height of the ring are in direct proportion to amount of albumin contained in the cerebrospinal fluid) and secondarily by persistence of a ring which appears more or less thick and dense in cases of acute meningitis and weblike in encephalic and medullary forms with scattered meningeal lesions. A negative reaction is declared to reveal itself by a total disappearance of the ring.

In a number of cases in which the test was applied by the author the reaction invariably yielded a positive result in acute meningitis while in other pathologic conditions of the central nervous system in which the principal lesions involved the nervous tissue in particular it demonstrated the altered composition of the cerebrospinal fluid.

Garrod L. P. On the Action of Certain Alleged Intestinal Antiseptics. *Brit. Med. Jour.* February 27, 1926, 367.

Method—About half a gram of feces is placed in a large sterile tube of known weight and accurately weighed. Sufficient sterile water is added to give a convenient dilution such as 1 in 50. Complete emulsification is carried out by forcing the fluid in and out of a long syringe with a wide bore needle (this process will do in two minutes what will occupy a mechanical shaker half an hour). Decimal or other convenient dilutions are then made from the emulsion and from each of the last two three or four dilutions 0.1 cc is sown on to large plates, previously dried thoroughly (at least 5 per cent of the water in the medium being evaporated by standing on a 55° C oven) and spread until the fluid sown has been absorbed by the surface of the medium leaving it dry. After incubation the colonies are counted, and the results expressed in millions of living organisms of each type per gram of feces.

Cultures made by this method yield uniformly spaced discrete colonies and apart from their value as affording quantitative results are superior to those made by the ordinary method.

The syringe employed for emulsification and the 0.1 cc pipettes for sowing had to be specially made for the purpose.

That the numerical accuracy of the results can be relied on was shown by duplicating the process, consistent results being obtained.

Four preparations—Dmol, Kerol, Yatren and Izol—described as intestinal antiseptics and given by mouth when examined by the procedure above were without appreciable influence on the number of living aerobic organisms in the feces.

Gross M. A Method of Reading Microreactions Macroscopically. *Klin. Wchn.* Feb. 19 1926, v, 342.

The following method was used in the performance of the Menecke flocculation test.

After the reagents are mixed in a glass dish a loopful of the mixture is taken up in a platinum loop measuring 0.04 cm in diameter and inserted in a cork. The cork is then placed in a test tube containing a small amount of wet cotton and may be incubated in a rack as desired.

In this perpendicular position most of the liquid collects in the lower third of the loop. Readings are made by holding the tube against a window and looking through the loop.

Campbell, W R Quantitative Determination of Dihydroxyacetone Jour Biol Chem, January, 1926, lxxvii, No 1, 59

Solutions

Dihydroxyacetone—A 1 per cent solution is prepared by dissolving 1 gm of dihydroxy acetone, previously kept in a desiccator, over phosphorus pentoxide until no further loss of weight occurs, and making up to 100 cc with distilled water. A few drops of toluene or of xylene are added and well shaken. The solution keeps a week.

Dihydroxyacetone 0.01 per cent solution—This solution containing 0.1 mg per cc is made by diluting 1 cc of the foregoing solution to 100 cc with distilled water in a volumetric flask. For the preparation of solutions containing 0.05 mg per cc and 0.2 mg per cc a 200 cc and a 50 cc volumetric flask is used respectively. A few drops of toluene are added. The dilute solution should be made daily.

KMnO₄ solution, 0.2 N—This is made up in the usual way, using 6.324 gm of pure potassium permanganate per liter of distilled water, allowing to age for a few days, filtering, and titrating against a known quantity of 0.1 N sodium oxalate solution with 5 cc of concentrated H₂SO₄ in a volume of 150 cc at 70° C.

KMnO₄ solution, 0.01 N—This solution is made fresh daily by diluting 0.2 N KMnO₄ solution to twenty times its original volume. Its titer should be carefully checked by test against an 0.01 N sodium oxalate solution, freshly diluted from 0.1 N sodium oxalate solution, to which 1 cc of 50 per cent H₂SO₄ has been added, and the solution kept at 70° C during titration.

Sodium Oxalate Solution, 0.1 N—The solution is made from United States Bureau of Standards sodium oxalate recently dried at 110° C for three hours and kept in a desiccator. Six and seven tenths gm of the pure dry salt are weighed out, dissolved, and made up to a liter with water. The addition of 5 cc of concentrated H₂SO₄ facilitates solution.

Sodium Oxalate Solution, 0.01 N—This is made by accurate tenfold dilution of the preceding solution of 0.1 N sodium oxalate.

Phosphate Molybdate Solution—To a liter beaker containing 35 gm molybdic acid and 5 gm sodium tungstate add 200 cc of 10 per cent sodium hydroxide and 200 cc of water. Boil vigorously twenty to forty minutes to expel ammonia, and cool. Dilute to about 350 cc, add 125 cc of concentrated (85 per cent) phosphoric acid, and dilute to 500 cc.

Colorimetric Method—After suitable dilution of the fluid containing dihydroxyacetone 2 cc of the diluted solution are mixed with 2 cc of the phosphate molybdate solution in a Folin Wu blood sugar tube and boiled in a water bath for fifteen minutes. The tube is then cooled in running water. The contents of the tube are diluted to 25 cc and compared in a colorimeter with the color developed from a suitable standard solution of dihydroxyacetone similarly treated. Standard solutions containing 0.5, 0.1, and 0.2 mg of dihydroxyacetone per cc furnish suitable standard colors. Standard solutions of glucose are not quite satisfactory owing to a slight difference in the quality of the colors.

Comment—The unknown should not be less than three fourths, nor more than one and one half times the strength of the standard, as the colors do not exactly match if these limits are exceeded.

When blood is being examined the tungstic acid filtrate as prepared by Folin and Wu is used. To 2 cc of oxalated blood 14 cc of water, 2 cc, of 10 per cent sodium tungstate, and 2 cc of $\frac{2}{3}$ normal H₂SO₄ are added in the order given, mixing after each addition, and well shaken and filtered after standing ten minutes. The filtrate is used undiluted in the method as described above, and a correction applied for the glucose and other reducing substances in the filtrate. When a standard set at 20 mm is used, the amount of the unknown is read off on the graph and 0.05 mg is subtracted. This purely empirical correction allows for glucose and other reducing substances in the filtrate.

Volumetric Method—Two cc of the suitably diluted solution are boiled fifteen minutes with an equal quantity of the acid phosphate molybdate solution and then cooled. The further procedure consists in reoxidizing the undiluted blue solution in the cold with 0.01 N

KMnO₄ solution from a recalibrated burette graduated in 1₅₀ c.c. One and fourteen hundredths c.c. of 0.01 N KMnO₄ equal 1/5 mg of dihydroxyacetone itself, or, using a Folin Wu blood filtrate, 114 c.c. of the permanganate solution equal 1 mg of dihydroxyacetone per c.c. of original blood

In carrying out this titration the permanganate solution is added slowly drop by drop, with shaking, to the cold blue solution until all blue color just disappears, leaving a colorless water clear solution. Titrating to the first pink tinge is unnecessary as the disappearance of the blue color furnishes a satisfactory end point. When the quantities of dihydroxyacetone are large, 1 c.c. of 4 N H₂SO₄ may be added to dissolve the oxide of manganese formed.

Scott W. J. and Leonard V. **Hexylresorcinol in the Treatment of Pyelitis of Infancy and of Childhood.** *Am Jour Dis Child*, February, 1926 xxxi 241

Report of a clinical study. The drug was administered as a 2.5 per cent solution, a teaspoonful (0.1 gm) t i d the dose being gradually increased to 0.2 or 0.3 gm t i d.

No toxic effects were noted. Occasional intolerance evidenced by cramps and diarrhea disappearing upon decrease or withdrawal of the drug. The dose may be continued indefinitely.

On the basis of clinical experience with hexylresorcinol in the treatment of pyelitis in children, it is believed that it is at times a distinct addition to the therapeutic measures heretofore avoided.

The striking improvement in the general health and nutrition frequently observed in children taking hexylresorcinol, does not seem to be entirely dependent on the control of the urinary infection, for it may occur long before there is any noticeable improvement in the local condition and is sometimes the most impressive effect of the treatment.

To obtain the best results, treatment should be persistent, the fluid intake should not be increased, and so should be avoided during administration of the drug.

Leonard V. and Froebisber M. **Clinical Application of Hexylresorcinol in Urology with Observations on the Significance of Surface Tension in Urinary Antisepsis.** *Jour Urol*, January, 1926, xv, 1

The successful application of hexylresorcinol in the treatment of chronic infections of the urinary tract depends therefore upon the strict observance of four factors all of which bear a distinct relationship to the surface tension of the urine.

1 The dosage must be adequate (0.6 grams three times daily). On smaller doses there may be insufficient reduction of the surface tension of the urine.

2 The fluid intake must not be increased for this not only dilutes the active hexylresorcinol in the urine but renders even that dilution less effective than it would be otherwise by raising the surface tension.

3 Sodium bicarbonate must be avoided for this drug raises the surface tension of the urine so markedly as to rob it of its bactericidal properties.

4 The course of treatment should be uninterrupted and sufficiently prolonged. The organisms which are most resistant to surface tension changes in the test tube (B. coli group) are most resistant to the action of hexylresorcinol in the urinary tract. Chronic B. coli infections ordinarily require from sixty to ninety days continuous treatment on doses of 0.6 gram (4 capsules) three times daily. Chronic coccus infections on the other hand may clear up completely with startling rapidity (forty eight hours) and ordinarily require less than three weeks treatment.

Elvehjem C. A. and Hart, E. B. **Quantitative Methods for the Determination of Iron in Biologic Materials.** *Jour Biol Chem* January, 1926, Lxxvii No 1, 43

Standard Iron Solution.—Dissolve 0.7 gm of ferrous ammonium sulphate (dried to constant weight) in 100 c.c. of distilled water and add 5 c.c. of concentrated sulphuric acid. Warm the solution slightly and add potassium permanganate until the iron is completely oxidized. Dilute the solution to 1 liter. One c.c. of the standard iron solution equals 0.1 mg Fe.

A 10 per cent solution of potassium thiocyanate

N/5 Potassium Permanganate—Dissolve 6.30 gm of the salt in distilled water and dilute to 1 liter

Hydrochloric Acid—Concentrated, free from iron

Nitric Acid—Concentrated, free from iron

Molybdate Solution—A solution of ammonium molybdate prepared in the usual manner on which a blank iron determination has been made to insure its freedom from iron

Forty per cent Solution of KOH—Prepared to be iron free by making a 40 per cent solution and allowing to stand for several days, decanting the iron free solution from the top

Procedure—A sample of the material to be analyzed is weighed out so as to contain between 0.1 to 0.3 mg of Fe. In the case of liquid milk 50 cc are used and evaporated to dryness. The sample is then carefully ignited in an electric furnace. A platinum dish is preferable for the ignition although a previously ignited and acid washed porcelain evaporating dish may be used successfully. The ash is taken up in about 10 cc of H_2O and 5 cc of concentrated HCl and allowed to stand for several hours. The residue is filtered off and the phosphorus removed from the filtrate in the usual manner, which consists of adding concentrated NH_4OH until the solution becomes cloudy, clearing up with concentrated HNO_3 in excess. Thirty cc of ammonium molybdate solution is added, digested on a water bath at $65^\circ C$ for one half hour, and the yellow precipitate of ammonium phosphomolybdate filtered off. The precipitate is carefully washed with dilute HNO_3 (9 cc HNO_3 in 100 cc H_2O) to insure the removal of the last traces of iron to the filtrate. Redigestion is not necessary as the small traces of phosphorus remaining in the filtrate will not interfere. The solution is brought almost to a boil and 40 per cent KOH (iron free) is added until no further precipitate forms, usually about 20 cc are required. The solution is boiled for several minutes to remove the ammonia present. The solution is allowed to cool and if the hydroxides do not settle properly a few cc of KOH are added and heated further. The precipitate is filtered off on an asbestos Gooch crucible, which has been carefully washed with HCl, by decanting with clear liquid first and finally adding the precipitate to the Gooch crucible. The precipitate is washed with very dilute KOH solution (1 to 2 per cent). Best results are obtained if only a low pressure is maintained on the suction flask during filtering. The precipitate is dissolved from the Gooch crucible with 25 cc of concentrated HCl, which is added in several portions (a few drops at a time) washing with water after each addition of acid. In this way the iron may be dissolved off completely and the total filtrate kept below 30 to 35 cc. The best method handling this small amount of solution is to introduce a test tube into the suction flask, allowing the end of the funnel to reach into the test tube so that the solution will be caught in the test tube instead of the suction flask. The solution in the test tube is then washed into the original beaker and the iron determined colorimetrically by adding enough N/5 $KMnO_4$ to produce a faint pinkish color (usually 1 to 2 drops) then adding 5 cc of a 10 per cent solution of potassium thiocyanate and making up to 50 cc volume in a volumetric flask. The color produced is compared in a Duboseq colorimeter with a standard color developed by taking 1 cc of the standard iron solution, adding 1 to 2 drops of N/5 $KMnO_4$, and 5 cc of the 10 per cent solution of potassium thiocyanate, and making up to 50 cc volume. The standard solution is set at 20 in the Duboseq and the unknown adjusted until the colors are equal. Since the standard contains 0.1 mg of iron, the amount of iron in the unknown is readily calculated. If the variation in the readings of the standard and unknown is too great a smaller or larger amount of the standard should be taken as the case may be.

Under certain conditions some difficulty may be experienced in filtering the precipitate of the hydroxide on the asbestos Gooch. This may be remedied by adding a small amount of ammonium oxalate (20 cc of 2.5 per cent solution) to the solution before addition of the KOH. Due to the presence of the oxalate the calcium will be precipitated as the oxalate, making the precipitate more crystalline and easier to filter. Of course, in this case, after filtering, the Gooch crucible must be dried and held in a flame for a short time to remove the oxalate or it will interfere with the color development. Upon ignition the cal

cium oxalate is changed to CaCO_3 , and if care is taken not to continue the heating long enough to form CaO it is easily dissolved in the amount of HCl we have suggested. We do not believe this scheme necessary as we have experienced no great difficulty in filtering in any of the determinations we have made. However, we have used this departure with success and it may be found to be useful in some cases.

Birkhaug K. E. Observations on the Etiology and Treatment with Erysipelas Antistreptococcal Serum. *Jour Am Med Assn*, May 8, 1926 LXXXVI 1411

Evidence is accumulating that erysipelas is due to a specific strain of the *Streptococcus hemolyticus*. Clinical trials in sixty moderately severe cases of erysipelas have demonstrated that intramuscular injection of erysipelas antistreptococcal serum, in amounts of 100 c.c. of unconcentrated, or 15 to 20 c.c. of concentrated serum, when administered during the first three days of the disease, causes a prompt lessening of the toxic depression, a critical fall in the temperature and pulse rate, prompt fading of the lesions and rapid absorption of the blebs and edema in the affected areas. In late cases the effect is strikingly favorable though repeated injections may be necessary. By means of a skin test dose of toxin injected intradermally and simultaneously with the intramuscular therapeutic dose, it is possible to observe whether complete neutralization of the circulating toxins is accomplished or whether an inadequate dose of serum was administered. It cannot be stated as yet whether the serum is purely antitoxic.

Neuhof H. and Cohen I. Abdominal Puncture in the Diagnosis of Acute Intraperitoneal Disease. *Annals Surg*, April 1926 454

Exploratory abdominal puncture in the opinion of the authors is a valuable diagnostic aid in obscure acute abdominal disease and should be employed in every obscure intraperitoneal lesion for which operation may be indicated.

The abdominal wall is prepared as usual and the skin either frozen or anesthetized with novocaine. A lumbar puncture needle to which a 10 or 20 c.c. syringe is accurately fitted is employed and a puncture made through the rectus muscle or near its outer border. The site of puncture can often be placed in the line of a probable laparotomy incision. To avoid carrying in bits of skin and to preserve delicacy of touch when the needle is introduced a small skin incision is made before the puncture. Aspiration is not employed over the site of a palpable or questionable mass nor in any acute or chronic intraabdominal disease in which a loop of intestine may be fixed.

The needle with stylet in place is introduced perpendicularly with a slow even pressure. When the peritoneal cavity is entered the syringe is attached and gentle suction maintained for several seconds and while the needle is being withdrawn. The fluid withdrawn, of which there may be only a drop in the lumen of the needle, may be examined in various ways and may furnish valuable information of diagnostic importance.

Duggan W. E. and Scott, E. L. A Critical Examination of Four Methods Commonly Used for the Determination of Sugar in the Blood. *Jour Biol Chem* January, 1926, LXVI, No 1, 287

The Fohn Wu and Hagedorn methods were found to be accurate and fairly precise when used to determine the concentrations of pure glucose solutions approximating those encountered in blood analysis.

It was found that an increase in the concentration of either the sugar or of the picric acid in the Benedict method would lead to high results, and this is offered as at least a partial explanation of the high results obtained by this method in certain pathologic bloods.

The Shaffer-Hartmann method was found to be reliable and satisfactory for concentrations above about 25 mg per 100 c.c. of blood. Certain corrections also appear to be necessary to their table. A substitute table is submitted.

Lash, A F A Comparison of the Incidence of Skin Reactions of the Toxins from Hemolytic Streptococci from Puerperal and Scarlet Fever Jour Am Med Assn, May 8, 1926, lxxxvi, 1427

A study was made of 247 women, 20 nonpregnant normal, 86 normal pregnant, 141 normal puerperal

The Streptococcus hemolyticus from puerperal fever is not one of the scarlet fever strains

A low incidence of positive reactions both to the Dick and puerperal toxins was encountered, probably due to inherent immunity The reaction to the two toxins in the same patient indicates that a person may be immune to one strain and not to another

Kulp, W L A Method for the Staining of Bacterial Flagella Stain Technology, April, 1926, 1, No 2, 60

A method is described with which nearly 100 per cent success has been obtained with nearly all species of bacteria

Reagents

Mordant Solution —

20 per cent solution of tannic acid..... 50 c c
(made by moistening 20 grams, transferring to a 100 c c flask and making up to volume with distilled water)

Cold, saturated solution of ferrous sulphate in distilled water 25 c c.

Saturated 95 per cent alcoholic solution of basic fuchsin,
Coleman and Bell..... 5 c c

Mix and allow to stand eighteen to twenty four hours The precipitate is removed by filtration through asbestos or by centrifuging The solution is then allowed to stand overnight in a Coplin jar and used without further filtering or centrifuging

Staining Solution —

Freshly filtered saturated solution of aniline water..... 100 c c

Saturated 95 per cent alcoholic solution of basic fuchsin.... 12 c c

Mix and allow to stand eighteen to twenty four hours, filter or centrifuge and transfer to Coplin staining jar

Preparation of Culture—A fresh agar slant having an appreciable amount of condensation water at its base is inoculated with the test organism and incubated at optimum temperature for twenty four hours At the end of this time a new slant is inoculated from the condensation water After twenty four hours' incubation of the second culture one or two drops of the condensation water (showing heavy growth) are poured aseptically into a large tube of sterile distilled water Tubes having a diameter of one inch and containing 15 to 20 c c distilled water offer a particular advantage here These water cultures are incubated at optimum temperature for from forty eight to seventy two hours As a rule, seventy two hours are preferable to shorter periods

After this incubation period a loopful of the water culture is transferred carefully to a clean glass slide The loop is filled by dipping into the culture without stirring or shaking, and transferred to the slide by touching the latter without drawing the loop over the surface or disturbing the suspension more than is necessary, as flagella are broken off easily in this stage of the procedure The drop on the slide is allowed to dry at room temperature or in a 37° C incubator No flame or heat higher than 37° C is employed for drying

Staining Method—1 Place the slide in the mordant solution for fifteen minutes at room temperature (20° 23° C) 2 Wash carefully in a slow stream of running water and shake off the excess water 3 Place in staining solution at room temperature for fifteen minutes 4 Wash as in 2 The precipitate on the under surface may be wiped off 5 Dry and examine

The method, *exactly followed*, has given excellent results with organisms of the colon typhoid group and several others

Kornhauser S I A Quick Iron Hematoxylin Method (as applied to fecal smears)
Stain Technology, April, 1926, 1, No 2, 78

A small part of the feces was rubbed up on a clean slide in physiologic salt solution and examined under the microscope for active protozoa. Then a drop of Donaldson's iodine eosin stain was added to bring out the cysts. This concluded the preliminary examination, and if the case were positive four permanent slides had to be made for confirmation and as a matter of record. Four clean slides were taken and numbered with a diamond to correspond to the patient's number in our daily record. A clean applicator stick was then used to obtain material which contained mucus or blood if such was present and streaked lightly on the slide. The slides were then dipped without drying into Coplin jars of hot Schaudinn's fluid, left there five minutes, washed in 50 per cent alcohol then 50 per cent alcohol plus iodine to remove any bichloride, 35 per cent alcohol then water. The wet slides were then placed on an electric hot plate and a 4 per cent iron alum solution was dropped on until the slides were covered and steaming. Any evaporation was taken care of by the further addition of iron alum from a pipette. This was continued for several minutes. Slides were then rinsed in water, put back on the hot plate and a ripe 0.5 per cent hematoxylin solution was added by means of a pipette the slide being kept full of liquid and steaming all the while. After several minutes they were placed in water differentiated in cold 2 per cent iron alum solution controlling the decolorization from time to time under the microscope. When the nuclei in the best parts of the smear stood out nicely decolorization was stopped and the slides washed in running water for at least five minutes. They were then dehydrated and mounted in balsam in the usual manner.

Thus within a half hour it was possible to have finished permanent slides with fine cytologic details shown to their best advantage. These preparations are now about seven years old and they are still as good as they were originally.

It might be added that experience with hematoxylin shows that black crystals would not do for this work, but that the white or light brown crystals made up in 10 per cent solution in alcohol and ripened and then added to water to make up 0.5 per cent hematoxylin would work nicely.

Burke V and Ashenfelter M Notes on the Gram Stain Stain Technology April
1926, 1, No 2, 63

1 The P_H of the environment in which organisms are grown may affect the Gram reaction.

2 Organisms should be classed as Gram positive or Gram negative according to the staining reaction when grown in the optimum P_H range for the species. Since the reaction may change with growth the media should be strongly buffered.

3 An alkali should be incorporated in the Gram staining technique in order to nullify the effect of an acid environment. We frequently wish to stain organisms from an environment not subject to control.

4 If alkali is added to the staining technique and the best American dyes are used the reaction of the medium upon which organisms are grown should not affect the Gram reaction.

5 There is no relation between acid and alkali production and the classification of bacteria regarding the Gram reaction. Acid and alkali producers are found in both groups.

6 The selection of a Gram staining technique should be based upon reliability of the methods under unfavorable conditions of acidity as well as on results obtained with borderline organisms.

There is included a discussion of the requirements of a practical Gram stain.

Hach T W Cultivation of the Gonococcus and Meningococcus Münch med Wchn
Feb 12 1926, lxxii, 275

Hach is enthusiastic over the efficiency of Hibler's medium prepared as follows:
Clean the brain from skins and mince it in the mincing machine. Strain it through gauze. Add an equal quantity of physiologic salt solution (85 per cent), stir well and fill

test tubes of 5 to 7 cc with the substance Sterilize for twenty minutes in the autoclave under 2 atmospheres pressure Over the 5 to 6 cm of a more or less homogenic sediment will be seen 1 cm of a liquid which is not always quite clear The reaction of this medium is acid, P_H 6.2 to 6.5

Miller, J W The Weigert Pal Technic for Staining Myelin Stain Technology, April, 1926, 1, No 2, 72

A method is described by which three difficulties of the original technic are overcome the tendency of the sections to become brittle, the difficulty of observing the reaction in the permanganate solution, and the slowness of the reaction

1 Formalin fixed material is embedded in celloidin and sections 75 to 90 microns are cut Sections of this thickness show the fiber tracts very well

2 Mordant in Muller's fluid in a warm place, for twelve to eighteen hours A paraffin oven at 35 to 39° C is best

3 Wash several times in distilled water

4 Stain eight to twenty four hours in Weigert Pal hematoxylin made as follows 10 cc of 10 per cent ripe hematoxylin solution in absolute alcohol 90 cc of distilled water 1 cc of 1 part saturated solution of lithium carbonate to 80 parts of distilled water

5 Wash in distilled water with a few drops (6 to 8 to 1000 cc water) of lithium carbonate for three to five minutes

6 Place sections in 50 per cent alcohol for a few minutes

7 Then in 80 per cent alcohol for ten to twelve hours This step makes the sections less brittle and the differentiation easier

8 Pass back through 50 per cent alcohol to water

In the following steps of differentiation two large Petri dishes containing the permanganate and oxalic acid solutions and a large crystallization dish containing distilled water are placed on the improvised glass hot plate, which illuminates the potassium permanganate solution and warms both the permanganate and oxalic acid solutions

9 Place sections in a 0.25 per cent aqueous solution of potassium permanganate until brown (one half to three minutes) The gray matter can be seen through the illuminated solution as a light brown

10 Rinse in distilled water one to three minutes

11 Place in a freshly prepared solution of equal parts of a 1 per cent aqueous solution of oxalic acid and a 1 per cent aqueous solution of potassium sulphite, to remove the brown color This should leave the myelin a dark blue to black color and the gray matter a pale gray or white If all the brown color does not come out, repeat the treatment with permanganate solution a short time The potassium sulphite must be fresh for good results

12 Wash in 3 changes of distilled water

13 Dehydrate through 50 per cent, 80 per cent and 95 per cent alcohols

14 Clear in orignum oil or better carbol xylol

15 Mount in neutral balsam

By this method as many as 300 sections may be stained and differentiated in the time it usually took to handle 50

REVIEWS

Books for Review should be sent to Dr Warren T Vanghan, Medical Arts Building,
Richmond, Va

*Neurological Fragments**

THE passing of a truly great man without contemporary biographical discussion or better—autobiographical contributions, constitutes a permanent loss to posterity. Opinion differs as to whether biography or autobiography is of greater value, but the greatest source of pleasure in the study of a man's life and activity comes from a reading of both and a comparison of those viewpoints expressed within.

Dr Jackson, known to all neurologists and best known to the medical profession in general as the first to describe what is now known as Jacksonian epilepsy, left no memoirs, and yet his life was one full of interest and his opinions served to direct in great degree subsequent neurologic progress. Dr Taylor has collected many of his scientific contributions those originally published by Dr Jackson over a long period of years under the group title *Neurological Fragments* and has published them with three biographical sketches. This combination of biography and the written word by which we may trace the writer's mental development, constitute a fair substitute for a true autobiography.

An Introduction to the Study of X Ray and Radium†

THE volume is based upon a series of demonstrations given at King's College Hospital and presents those facts concerning x rays and radium which will afford the reader an idea of their nature and application. The technique of clinical examination and the interpretation of findings are not included. The book covers the knowledge in pure and applied physics relating to radiology which must of necessity form the groundwork of a comprehensive understanding of roentgenology. We find chapters on radioactivity, the preparation of radium salt, the preparation and measurement of radium emanation, the action of radium upon normal living tissues, the atomic theory, methods of protection against the x ray and radium, and notes on x ray and radium therapy. The historical description of the development of clinical radiology is of particular interest.

It is with some interest that we note that the authors do not recommend x ray treatment as a sole procedure in operative malignancy. They feel that where there is a possibility of cure from surgical extirpation, this should be done. Of course x ray therapy is used in conjunction with surgery.

The volume should be recommended to all radiologists, to undergraduate students at the beginning of their acquaintanceship with roentgenology and to those practitioners who are referring their work to the roentgenologist and wish to have a first hand acquaintance with what is actually being done.

Neurological Fragments. By J Hughlings Jackson M.D. F.R.S. F.R.C.P. with biographical memoir by James Taylor M.D. F.R.C.P. and including the Recollections of the late Sir Jonathan Hutchinson and the late Dr Charles Mercier. Cloth. Oxford Univ. Press 1915.

†*An Introduction to the Study of X rays and Radium.* By Hector A. Colwell M.B. (Lond.), D.P.H. (Oxf.) and Cecil P. G. Wakeley F.R.C.S. (Eng) F.R.S. (Edin.) Cloth. Price \$3.35 Pp. 63 Illustrated. Oxford Univ. Press 1926.

NOTE. Insofar as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Bacteriology and Applied Immunology for Nurses[†]

BEGINNING with a brief history of the science of bacteriology the author proceeds with a description of bacteria in general and their classification, methods of cultivation, examination and staining, and methods for their destruction

After pointing out that most bacteria are not disease producers, he outlines briefly the mode of production of disease by bacterial infection, describes the bacteriologic characteristics of the more important bacteria and proceeds with discussion of the mechanism of infection. This carries us naturally to the subject of immunity and the mechanism of its production. The author selects Ehrlich's side chain theory as suitable for presentation and the most easily visualized and comprehended. At the same time he wisely italicizes the remark that the illustrative figures have no actual counterpart in the cells of the body and are purely imaginary.

An understanding of the general principles of immunity having been acquired, the work now proceeds with discussion of their practical application in applied immunology. The nurse becomes acquainted briefly with the agglutination test in typhoid, dysentery, pneumonia, and in blood grouping, with the luetin test, the tuberculin test, the Schick test, the pollen reactions and the complement fixation test. Following these procedures which are of value in diagnosis there is a section devoted to immunologic methods of treatment including the preparation and use of serums and a discussion of the phenomenon and treatment of anaphylaxis.

We find chapters on the bacteriology of milk, water, and foodstuffs, the collection of specimens for laboratory examinations, a most important chapter too often not understood by the present day nurse, chapters on protozoa, etc.

The introduction is excellent. It stresses the knowledge that the function of the nurse is to assist nature. The author points out that because of her intimate relationship with the patient and the public, her part in the education of the people at large can be and must be of great importance. She should be well prepared to give an intelligent reason for the use of vaccines and serums and know what they are and how they act, what they will and will not do, how vaccination will serve as a preventive against smallpox and typhoid fever and how to answer and confute objections to the use of these methods. "The ability to do this represents the difference between a fully trained and equipped nurse—a real guardian of the public health—and a mere attendant, trained more or less in the mechanical duties of the sick room."

After having read the introduction one wishes that it might be reproduced in toto at the end of volume for it is after having become acquainted with the science of bacteriology and immunology that the nurse can first truly appreciate the remarks of the introduction. However, the author has improved on this idea for the closing chapters of the volume deal with the prevention of disease, the immunization of the individual, applied sanitation, vivisection and the education of the public.

The illustrations are excellently done and the physical craftsmanship of the volume is unusually good.

Midwifery Mechanics[†]

THIS is not simply a text on the mechanical principles involved in the successful delivery of a living child in the various presentations, but is a monograph on the author's conception of the fundamental mechanical principles of parturition.

Since the time of Hippocrates obstetricians have sought out ways and means of lightening the labor of a parturient mother. So much so that nowadays whenever a novel suggestion is put forth, such as twilight sleep or a recently popularized form of version, our first reaction is of an adverse nature and our first question is as to the ultimate result, particularly the maternal or infant mortality.

*Bacteriology and Applied Immunology for Nurses. By Robert A. Kilduff, A.B. A.M. M.D. Director Laboratories Atlantic City Hospital. City Bacteriologist Atlantic City N. J. Consulting Serologist Providence Hospital, Beaver Falls Penna. Major Medical Corps Altho Leather Illustrated Price \$2.00 Pp 252 The Bruce Publishing Company Milwaukee 1926

†Midwifery Mechanics. By Lieut.-Colonel Andrew Buchanan I.M.S. (Retd.) M.A. M.D.-M.Ch., M.A.O. Cloth Price \$2.50 Pp 82 Illustrated. Oxford Univ. Press First printed 1924

Dr Buchanan, however, offers no startlingly new methods designed to improve on those already found so successful by nature

His first and foremost objective is to explain the mechanism of normal delivery in terms that will be more readily understood by the students of obstetrics. His explanation is certainly original and is readily understood, particularly so since the monograph is abundantly and excellently illustrated with diagrammatic sketches and with photographs of his illustrative mannikins. The principle on which the entire discussion is based is the need for juxtaposition of two pivot points, one in the female pelvis and one in the fetus. When these two points are close together delivery becomes a simple matter, when they are widely separated, trouble ensues. The space available in this review does not permit a more detailed exposition of this theory of pivot points.

Where the pivot points are widely separated so as to interfere with satisfactory rotation of the head through the pelvis around the pubic symphysis as an axis, the author describes the methods best calculated to procure closer approximation.

The volume should appeal to curious minded obstetricians rather than to obstetric faddists.

*Pulmonary Arteritis**

THE volume, written in Spanish, presents in an interesting fashion an original study of sclerosis of the pulmonary artery.

The purpose of the volume has been adequately set forth. The author with some of his coworkers, maintains that sclerosis of the pulmonary artery exists as a primary affection in which syphilis plays the most important role as a causative agent and that the disease also exists as a clinical entity capable of being diagnosed.

The earliest symptoms are cyanosis and dyspnea. Cyanosis manifests itself first, being marked and localizing itself principally in the face. The extremities however are also strikingly affected. It is this striking cyanosis that led Ayerza to name the disease "Cardiacos negros."

Dyspnea follows months at times years, later. The interesting thing about this symptom is that it may go on indefinitely in the absence of the other mechanical concomitants, that is, edema, hepatic congestion, congestion of the lungs etc. These however with cardiac insufficiency appear later and may last several years.

A hitherto eptysis presents itself with a certain regularity in these patients.

Headaches are of frequent occurrence having no exact localization. They are very constant and mostly nocturnal.

Faintness and giddiness coming in spells are symptoms.

Finally, a strange symptom that appears in the late stages of the disease is hyper somnia.

The author has surveyed and reviewed the material and literature on the subject systematically and with thoroughness. His investigations are clearly presented and the anatomic, histologic and pathologic studies that he includes help the reader to obtain a more comprehensive picture of the lesion found in this disease.

It is interesting to note that the so called 'black cardiac cases' are synonymously known by many names, and because the author believes that the arterial process involved is an inflammatory reaction, he has purposely chosen the title *La Arteritis Pulmonar* for his volume.

Though the author maintains that pulmonary arteritis exists as a primary disease and that certain cardiac diseases and chronic pulmonary diseases are only factors of a secondary nature, the reviewer feels that where syphilis is not definitely found the evidence is not entirely satisfactory that it is a primary disease and that here cardiac and chronic pulmonary diseases play more than a secondary role in its etiology.

It is an admirable piece of work, one giving the clinician or diagnostician valuable information and since the author has put down a definite line of symptoms for its manifestation, it should be of importance in the field of differential diagnosis.

*The Diagnosis, Treatment and End-Results of Tuberculous Disease of the Hip Joint**

THE author sets forth his thesis so clearly in his introduction that we quote him at some length

"In discussing diagnosis attention has been focussed on the diagnosis of the early case. Our aim should be the recognition of the presence of tuberculous disease at the earliest possible moment. Consequently, no mention is made of the former customary division of the disease into three stages, only the signs and symptoms of early disease are commented upon.

"The treatment meted out for tuberculous disease of the hip joint has varied from decade to decade. Amputation, which was at one time the sole resort of the surgeon, was superseded by the operation of excision of the head of the femur. When the poor results following excision became known, the operation was discarded, and the joint treated by local nonoperative measures. The exact lines of treatment varied, the American school, championed by Sayre, believed in Traction, the English school under Thomas pinned their faith to Fixation. Then attention was directed to the fact that tuberculosis was a disease affecting the whole of the body, and that the joint condition was a local manifestation of a general condition, and open air treatment, heliotherapy, and vaccines were brought to the aid of the surgeon. At the present time the popular method of treatment in England is non operative local treatment (recumbency, traction, and fixation) combined with general treatment (open air, hygiene and heliotherapy). This line of treatment is described in detail in this paper."

He emphasizes that the end results are not represented by the condition at the time of discharge from the hospital but by the condition years later, after the joint has had to stand the wear and tear of normal life. He further emphasizes that the exact location of the disease in the hip joint is an important factor in determining the end results. Disease of the acetabulum differs radically from the disease of the head of the femur and this again from synovial disease or disease affecting the neck of the femur.

Fortschritte der Heilstoffchemie (Advance in the Chemistry of Therapeutic Substances)†

THIS is the first of a series of nine volumes on the literature of physiologically active substances. The work will be divided into two parts, part one will cover the patents which have been taken out on such substances, and part two the scientific literature. This is the first of six volumes on the patents and covers the German patents from 1877 to 1900. It is very well arranged so that it is easy to find any patent or any substance desired. In the first 84 pages the patents are arranged according to the classes and groups of the German patent office. This brings together all the patents on any particular subject. A short statement is made concerning the content and purpose of each patent and at the end of each group is a longer statement about the chemistry and the physiologic activity of the members of the group.

In the following 886 pages which make up the body of the book the patents are arranged in numerical order and the texts of the patents are given in full, together with the chemical formulas, drawings of apparatus, etc. Then follows an index of the patentees and a very complete subject index which includes many of the trade names of the patented substances.

It is obvious that the volume contains a mine of useful information conveniently arranged.

*The Diagnosis Treatment and End-Results of Tuberculous Disease of the Hip Joint. By George Perkins M.Ch. (Oxon.), F.R.C.S. Eng. Assistant Surgeon to the Royal National Orthopedic Hospital. Assistant Surgeon to Pyrford Orthopedic Hospital. Orthopedic Registrar St. Thomas Hospital. The Robert Jones Prize Monograph 1924 published under the auspices of the British Orthopedic Association. Cloth Price \$1.75 Illustrated Pp 118 Oxford Univ. Press 1926.

†Fortschritte der Heilstoffchemie (Advance in the Chemistry of Therapeutic Substances). By J. Houben Professor of Chemistry in the University of Berlin. Part I German patents, Walter de Gruyter and Co. Berlin 1926 80 marks.

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO, JANUARY 1927

No 4

Editor in Chief WARREN T VAUGHAN, M D

Richmond, Va.

ASSOCIATE EDITORS

DENNIS E JACKSON M D	CINCINNATI
PAUL G WOOLLEY M D	LOS ANGELES
J J R MACLEOD M B	TORONTO
W C MACCARTY, M D	ROCHESTER MINN
GERALD B WEBB, M D	COLORADO SPRINGS
VICTOR C MYERS PH D	IOWA CITY
RUSSELL L HADEN, M D	ROSEDALE ILL
JOHN A. KOLMER M D	PHILADELPHIA
ROBERT A. KILDUFFE M D	ATLANTIC CITY N J
A. PARKER HITCHENS M D	MANILA P I
DEAN LEWIS, M D	BALTIMORE
GEORGE E HERRMANN M D	NEW ORLEANS
HAROLD D CAYLOR M D	ROCHESTER, MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis Mo as Second Class Matter

EDITORIALS

Sclerosis of the Pulmonary Artery

SO INFREQUENT is pronounced sclerosis of the pulmonary artery that for a time it was thought that sclerosis was an affection of the greater circulation only. Andral in 1829 first described atheromatous fibroid or cartilaginous plaques in the pulmonary artery. Bouillaud in 1839 found similar changes in a young child with mitral stenosis. Since then the coincidence of pulmonary arteritis and mitral stenosis has been repeatedly remarked and has been explained as a result of increased tension in the lesser circulation caused by ventricular hypertrophy. Dittrich (1850), Bamberger (1857), Traube (1878) likewise described pulmonary arteritis with coincident mitral stenosis.

Others later found the lesion without accompanying gross cardiac pathology. Then, naturally, the infection intoxication hypothesis was proposed. Sanne (1877) found the site of predilection at the arterial bifurcations and

be double the diameter of the aorta, increased size of the heart shadow to the right, arborization shadows extending out into the lung parenchyma

The pathology in primary arteritis consists of a generalized sclerosis extending into the smallest arteries, which are at times obliterated, with dilation of the main arterial trunk and cardiac alterations as described above. Arteritis and thrombus formation are found especially in the large and medium-size vessels. The systemic circulation shows no evidence of sclerosis.

While Waithin has established that spirochetes may exist in the walls of the pulmonary arteries and Elizalde and Arrillaga found them in a case of pulmonary arteritis complicated by syphilitic bronchopneumonia, Vaquez concludes that the evidence is insufficient and cannot be accepted as final and that while syphilis may be a factor, the cause of idiopathic sclerosis of the pulmonary artery is still obscure.*

REFERENCES

- Vaquez, H. Sclerose de l'artere pulmonaire, Paris Medical, July 3, 1926, vii, 15
 Arrillaga, F. C. La Arteritis Pulmonar y suyo Cuadro Clinico, Buenos Aires, 1926
 —W T V

The Reviews

OF what use are book reviews? Of what value may they be? Perhaps the least useful purpose to which a review may be put, is as a critical investigation, with the idea of pointing out errors and fallacies (usually unimportant ones), to the apparent aggrandizement of the reviewer.

The reader of a review is, first, interested to know the contents of the book under consideration so that he may decide whether to invest therein. The title is on a subject in which Dr. Jones has been rather interested. How closely does the volume follow the indications of the title? Is the treatment elementary, a textbook discussion, or monographic? Has the author written his text for the benefit of the novice or is it highly technical, for the perusal of those whose thoughts and studies have followed similar channels? How well will it fulfil Dr. Jones' requirements?

The printing of book reviews is rather costly and the space devoted thereto is sorely needed. The reviews must therefore justify their existence. To justify their existence, they must be read. To be read they must present to the reader some item or items of information, previously not known by him, —knowledge which he is glad to have for future use.

In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

—W T V

*Further discussion of the clinical symptomatology of this condition (Arrillaga) will be found in the book review section of this Journal.

for years have had chronic recurrences of bronchitis, with exacerbations and resulting pulmonary emphysema. Often the provocative cause is a chronic fibroid phthisis. Eventually, however, the dyspnea which has previously existed only with the acute attacks, becomes permanent and thus, as in the cardiac cases, is followed by progressing cyanosis. Only later do symptoms of congestive heart failure become manifest.

The postmortem picture in sclerosis consecutive to pulmonary infection, while closely resembling that following a long standing mitral stenosis, is likely to show a more patchy distribution.

In both cases the heart shows distinct thickening of the right ventricular wall, often greater than the thickness of the left. During life roentgen examination shows an increase in the transverse diameter of the heart, particularly toward the right, an increased pulmonary arborization, with exaggeration of the hilar shadows, and pronounced visibility of the arborization and ramifications of the pulmonary vessels.

While sclerosis is the dominant feature following mitral stenosis and has been attributed to long continued hypertension in the lesser circulation, the type which is dependent on chronic pulmonary inflammatory processes appears to be more of a true arteritis, the vessel walls showing inflammatory foci, thromboses, even mural vegetations. The scattered distribution of these lesions indicates a subacute pulmonary arteritis progressing toward generalized sclerosis.

Vaquez insists, however, that the fundamental cause is the same in both. He recognized the predisposing effect of hypertension but believes that in the cardiac cases also, infection is the ultimate cause of the sclerosis, infection more easily acquired in a vessel impaired by long standing hypertension. He believes that the occasional attacks of hemoptysis are associated with atheromatous plaques.

Of chief interest are the cases of primary or idiopathic sclerosis. Vaquez describes two such cases, one of which was correctly diagnosed radiographically during life. Only three cases are on record in which the diagnosis was established before the death of the patient. The clinical history is as described above but none of the predisposing causes are present. There is no cardiac disease and the lungs are clear of all forms of infection. As we have stated above, Rogers believed that syphilis was the cause of those cases reported by him. Arrillago, whose recent book on pulmonary arteritis is reviewed in this number of the *Journal*, likewise believes that syphilis is a most important factor. Vaquez noted some improvement in his case following antisyphilitic treatment but the blood Wassermann was negative and the patient gave no history of venereal disease. Thus while the spirochete is still under suspicion, it has not as yet been found guilty.

Idiopathic pulmonary arteritis has been reported chiefly in young individuals ranging from twelve to forty seven years. There is usually an increase in the red cell count. Not infrequently anginal symptoms follow exertion.

Diagnosis is established on radioscopic examination which shows dilation of the pulmonary artery at its origin, sometimes so pronounced that it may

be double the diameter of the aorta, increased size of the heart shadow to the right, arborization shadows extending out into the lung parenchyma

The pathology in primary arteritis consists of a generalized sclerosis extending into the smallest arteries, which are at times obliterated, with dilation of the main arterial trunk and cardiac alterations as described above. Arteritis and thrombus formation are found especially in the large and medium-size vessels. The systemic circulation shows no evidence of sclerosis.

While Warthin has established that spirochetes may exist in the walls of the pulmonary arteries and Elzalde and Arrillaga found them in a case of pulmonary arteritis complicated by syphilitic bronchopneumonia, Vaquez concludes that the evidence is insufficient and cannot be accepted as final and that while syphilis may be a factor, the cause of idiopathic sclerosis of the pulmonary artery is still obscure.*

REFERENCES

- Vaquez, H. Sclerose de l'artere pulmonaire, *Paris Medical*, July 3, 1926, vi, 15
 Arrillaga, F. C. La Arteritis Pulmonar y suyo Cuadro Clinico, Buenos Aires, 1926

—W T V

The Reviews

OF what use are book reviews? Of what value may they be? Perhaps the least useful purpose to which a review may be put, is as a critical investigation, with the idea of pointing out errors and fallacies (usually unimportant ones), to the apparent aggrandizement of the reviewer.

The reader of a review is, first, interested to know the contents of the book under consideration so that he may decide whether to invest therein. The title is on a subject in which Dr. Jones has been rather interested. How closely does the volume follow the indications of the title? Is the treatment elementary, a textbook discussion, or monographic? Has the author written his text for the benefit of the novice or is it highly technical, for the perusal of those whose thoughts and studies have followed similar channels? How well will it fulfil Dr. Jones' requirements?

The printing of book reviews is rather costly and the space devoted thereto is sorely needed. The reviews must therefore justify their existence. To justify their existence, they must be read. To be read they must present to the reader some item or items of information, previously not known by him, —knowledge which he is glad to have for future use.

In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

—W T V

*Further discussion of the clinical symptomatology of this condition (Arrillaga) will be found in the book review section of this Journal.

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D. Editor in Chief
Medical Arts Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.D.
University of Toronto Toronto

Clinical Pathology

W. C. MacCARTHY, M.D.
Mayo Clinic Rochester Minn.

Biochemistry

VICTOR C. MYERS, Ph.D.
State University of Iowa Iowa City

Immunology

JOHN A. I. OLIVER, M.D.
University of Pennsylvania,
Philadelphia

Bacteriology

A. PARKER HITCHENS, M.D.
Bureau of Science Manila, P. I.

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

Pathology

PAUL G. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD B. WELLS, M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR, M.D.
Mayo Clinic Rochester Minn.

Experimental Medicine

REUEL L. I. HADEN, M.D.
University of Kansas Rosedale

ROBERT A. KILDUFF, M.D.
Atlantic City N. J.

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSEBY COMPANY 3523 25th BLVD. ST. LOUIS
Entered at the Post Office at St. Louis Mo. as Second Class Matter

LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment. Estimates cheerfully furnished Prompt Service

See our advertisements pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA, PA.

[NUMBER FIVE OF A SERIES]

Little Glimpses

OF THE SCIENTIFIC AND RESEARCH WORK
OF PARKE, DAVIS & COMPANY



CHEMICAL ANALYSES

Hundreds of tests are made in our analytical department every week in order to insure the purity and strength of the various chemicals used in manufacturing operations, and also to determine the uniform activity of finished preparations

PARKE, DAVIS & COMPANY

Home Offices and Laboratories

DETROIT, MICHIGAN

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO FEBRUARY 1927

No 5

CLINICAL AND EXPERIMENTAL

BLOOD GROUPS IN GENERAL PARALYSIS

A STUDY OF THE AGGLUTININ AGGLUTINOGEN FORMULA IN NINETY ONE CASES*

BY HENRY A BUNKER, JR, M D AND SIDNEY MEYERS, NEW YORK, N Y

THERE is a considerable body of evidence at hand that the isoagglutinin content of human blood is a constitutional character. The four isoagglutinin groups traditionally derivable on the basis of the presence or absence of agglutinable substances *a* and *b* (Jansky¹ & Moss²) occur as fixed biochemical conditions, with an incidence which varies rather definitely with different races³ and which is not susceptible to alteration by environmental influences⁴. There is considerable evidence moreover, that the presence or absence of the isoagglutinable elements *a* and *b* is an hereditary quality, transmitted from parent to offspring. It is interesting that the precise mechanism of this transmission has recently come into dispute whereas it has long been assumed to involve independent pairs of factors (the agglutinogens *a* and *b* being dominant to their respective isoagglutinins *A* and *B*), Bernstein⁵ has recently suggested that the blood groups are inherited as a series of three multiple allelomorphs (the agglutinogens *a* and *b* being both dominant to the same recessive (*R*) thus taking the agglutinins out of hereditary control and leaving only the agglutinogens to be inherited), and Snyder⁶ after an analysis of all the matings so far published, including a particularly extensive series of his own, concludes that the older hypothesis must be discarded.

Whether or not constitutional factors are the basis of the proclivity on the part of a small proportion of individuals previously infected with syphilis to develop parenchymatous syphilitic involvement of the central nervous system (general paralysis) is a question which we are now investigating from certain standpoints. Incidental to this work we have paused to determine

From the New York Psychiatric Institute Ward's Island New York.
Received for publication Sept 6 1926

the isoagglutinin groups to which a series of ninety-one patients suffering from general paralysis belonged, in order to discover whether the incidence of the four blood groups among these patients differed from that of the population at large, or—what is the same thing, as Straszyński⁷ has recently shown—from that of syphilitics in general. It must be admitted, however, that no correlation between blood group and any constitutional disorder has so far been demonstrated, in no instance thus far recorded has the incidence of the blood groups among the subjects of a given disease differed decisively from that of the general population. Hirszfeld and Brokman,⁸ for example, have shown that although susceptibility or immunity to diphtheria is, like the isoagglutinin content of the blood, an inheritable and constitutionally conditioned character, the isoagglutinin “types” nevertheless do not differ in immunity to diphtherial infection. “susceptibility or immunity to diphtheria is not closely connected with either of the biochemical properties of the blood.” The absence of any linkage of this character has been demonstrated likewise for syphilis,⁷ and by Snyder³ for dementia precox, epilepsy and mental deficiency, only in the case of cancer is some doubt perhaps permissible, since Weitzner,⁹ and still more recently Bendien,¹⁰ have both reported finding Group IV (Moss) represented among carcinomatous subjects to a smaller degree, and Group I to a greater degree, than in normal persons. Hence the possibility that patients with general paralysis might differ from other syphilitics in this respect appears rather a remote one, in spite of the moderate degree of correlation which Straszyński⁷ has reported to exist between the blood group and the persistence of the Wassermann reaction under treatment. Nevertheless, it seemed of interest to determine the isoagglutinin groups to which the patients in our series belonged, even though in the end this procedure might be found to have afforded only the opportunity to corroborate the findings of certain workers who have demonstrated the apparent existence in human blood of isoagglutinins other than the traditional two (*a* and *b*) first postulated by Landsteiner.¹¹

PRELIMINARY CLASSIFICATION

For the carrying out of agglutination tests by the microscopic method, blood was drawn from the vein, the serum pipetted off after standing over night, and the corpuscles, obtained by mixing about 0.5 cc of blood with 1.5 per cent sodium citrate in normal salt solution, centrifuged and washed three times with normal saline solution. Hanging-drop preparations were then set up in duplicate in the usual manner, using two loopfuls of serum and one loopful of a 1 per cent suspension of corpuscles in salt solution. The preparations were examined at the time, and at the end of one and two hours, and in the case of negatives at the end of four hours as well, during these intervals they were kept in the ice box at 5° to 10° C, on account of the fact, well brought out by Guthrie and Pessel,¹² that an amount of agglutinin sufficient to cause marked agglutination in the ice box may escape recognition in tests made at 37° C or at room temperature. Autoagglutination (said to be a rare phenomenon in human bloods), which takes place only at low temperatures and might accordingly lead to confusion, was readily excluded by a

preliminary matching of the serum of each patient against his own corpuscles, only two instances of autoagglutination in the series were discovered, in each the autoagglutinin was removed from the serum, prior to its further use, by absorption with the corpuscles of the same individual.

All tests were performed by matching both the unknown serum and the unknown corpuscles against known corpuscles and known serum, respectively, in accordance with the schema shown in Table I.

TABLE I

SÉRUM		x RED CELLS			
Group II	+	0	+	0	
Group III	+	+	0	0	
Assigns x to	Group I	Group II	Group III	Group IV	
Scrum		Red Cells			Assigns x to
		Group II	Group III		
x		0	0		Group I
x		0	+		Group II
x		+	0		Group III
x		+	+		Group IV

The group numbering of Moss is here employed rather than the now official nomenclature of Jansky for the sake of uniformity with Guthrie upon whose work the present investigation is based.

The results thus checked against each other are presented in Table II which gives the distribution of the four traditional blood groups among the ninety one general paralytics in this series as compared with their distribution among the population at large.

TABLE II

	91 PATIENTS WITH GENERAL PARALYSIS	GENERAL POPULATION		
		7 000 EUROPEANS (HIPSZVEID)	1 122 AMERICANS U. S. ARMY (MOFFITT)	5 000 AMERICANS (CULPEPPER)
Group I	4.5	4.0	4.8	5.2
Group II	41.5	41.4	31.6	30.0
Group III	14.5	11.3	6.2	14.3
Group IV	39.5	42.8	55.4	44.5

From the racial standpoint it might be mentioned that our material was made up of 31 Jews (5 American born) and 70 Gentiles (4 American born) all were Caucasians except four negroes two of whom were members of Group III.

It is thus apparent that the patients in this series do not differ essentially from the general population with reference to the percentage claimed by each of the four blood groups. General paralysis then no more than syphilis itself, is characterized by any difference from the population at large in the incidence of the blood groups which it exhibits.

GROUP II CASES

In an admirable series of papers which we may warmly recommend to the reader as a most able consideration of the entire subject, Guthrie and Huck¹² have pointed out that 'the methods generally employed for blood grouping are based upon the assumption that there are only four isozym glutin groups and that the blood of every human being belongs to one

of these groups. The tests based on this assumption," they go on to say, "are so devised as to cause each blood tested to fall into one or other of these four groups, thus serving to perpetuate a belief which no one has seriously questioned." They then present evidence that "the popular belief concerning the existence of four and only four isoagglutinin groups is incorrect" through the demonstration, by direct tests and by absorption experiments, of the existence of at least a third isoagglutinin and a third isoagglutininogen.

Without following out each step which marked the pioneer work in this direction of Guthrie and his coworkers, we may summarize their results, first, as obtained among the members of "Group II."

They found that, in the first place, the erythrocytes of their various "Group II" individuals did not behave alike. For, briefly stated, Group IV serum, after absorption with the cells of a Group I individual, no longer agglutinated the cells of Group I, Group III, and certain of the members of "Group II," *but did agglutinate the cells of other members of "Group II,"* and when absorbed with the cells of the first-mentioned members of "Group II," lost (naturally) the power to agglutinate the cells of these individuals, *but still strongly agglutinated the cells of the other members of "Group II"* (as well as the cells of Group I and Group III).

TABLE III

SERUM	SUBSEQUENT AGGLUTINATION OF RED CELLS			
	GROUP I	GROUP III	GROUP II _a	GROUP II _β
1 Group IV	+	+	+	+
2 Group IV (after absorption with cells of Group I)	0	0	0	+
3 Group IV (after absorption with cells of Group II _a)	+	+	0	+

Hence there may be assumed the existence of an agglutininogen (*b*) common to both types of "Group II" individuals (Table III 1 vs 2 and 3), and likewise an additional agglutininogen (*c*) present in the erythrocytes of Group II β but not in those of Group II α (Table III 2 and 3). This necessitates, obviously, the presence of the corresponding agglutinin (*C*) in the serum of Group IV.

The phenomena represented in Table III may be schematized as follows:

Table III 1	ABC ⁺ -	cross agglutinated with	(gr IV serum)	-ab	= +
				(gr I cells)	
				-a	= +
				(gr III cells)	
				-b	= +
				(gr II _a cells)	
				-bc	= +
				(gr II _β cells)	

*Since Group IV serum agglutinated the cells of Group II and Group III (Table III 1) this serum must contain the agglutinins (1 and B) corresponding to the agglutininogen (*b*) present in the cells of Group II and to the agglutininogen (*a*) present in the cells of Group III. For it will be remembered that Group II and Group III are reciprocally agglutinative and the sera of both agglutinate the corpuscles of Group I (Table I). Hence we can sum up in the accepted manner the presence in the cells of Group III of an agglutininogen (*a*) corresponding to the agglutinin (*A*) in the serum of Group II and in the serum of Group III in agglutinin (*B*) corresponding to the agglutininogen (*b*) in the cells of Group II. Therefore in the cells of Group I both the agglutinogens (*a* and *b*) possessed by the cells of Group II (*b*) and Group III (*a*).

†For convenience the elements which take part in absorption or agglutination are designated in heavy-faced type.

Table III	2	ABC- (gr IV serum)	absorbed with (gr I cells)	-ab	→	C- (absorbed serum)
						-ab = 0 (gr I cells)
						-a = 0 (gr III cells)
						-b = 0 (gr II α cells)
						-bc = + (gr II β cells)
		C- (absorbed serum)	cross agglutinated with			

Table III	3	ABC- (gr IV serum)	absorbed with (gr II α cells)	-b	→	AC- (absorbed serum)
						-ab = + (gr I cells)
						-a = + (gr III cells)
						-b = 0 (gr II α cells)
						-bc = + (gr II β cells)
		AC- (absorbed serum)	cross agglutinated with			

It therefore follows that Group IV serum from which agglutinin *B* has been removed by absorption with Group II α cells will thus serve to distinguish between the α and β members of Group II¹¹, for agglutination of the II α cells, which contain only agglutinin *b*, will not be affected by a serum lacking agglutinin *B*, whereas agglutination of the II β cells which contain agglutinin *c* as well as *b*, will be readily brought about through the presence in the absorbed serum of agglutinin *C*.

It was accordingly a simple matter to follow the procedure outlined by Guthrie and Huck,¹³ of absorbing serum from successive members of Group IV with erythrocytes from successive members of Group II¹¹ until a serum was found which would completely agglutinate the corpuscles of an individual apparently belonging to Group II after that serum had been entirely deprived of agglutinins for the corpuscles of other individuals classified in the same group*. Having found such a group IV serum, it was needful only to perform cross agglutination tests with the corpuscles of all the "Group II" patients in our series to determine which of them had the agglutinin formula -*b* and which the formula -*bc*.

In this way it was discovered that of 37 patients in "Group II," 29 possessed the additional agglutinin *c* and 8 were without it, that is, 78 per cent of the group contained the extra agglutinin in their corpuscles.

Guthrie and Huck¹³ expressed themselves only tentatively at first with regard to the relative incidence of these two formulae within "Group II," believing the formula -*b* to be commoner than -*bc*. Later however, they were led to question the accuracy of their earlier impression concerning the relative frequency with which these two types are encountered within "Group II."¹⁴ Within the year, Coea and Klein¹ furthermore described an additional agglutinable substance which they termed *Y* and which they believed to be

¹⁴ In performing the absorption experiments we found it often advisable to employ a larger volume of corpuscles in proportion to serum than did Guthrie and Huck—about 10 volumes of packed corpuscles (a .15 per cent suspension) to 15 volumes of serum rather than 9 volumes of the former to 10 volumes of the latter. Like these writers we carried out all our absorptions in the ice box but often for four to six hours rather than for two hours.

possibly identical with the agglutinin *c* of Guthrie and Huck, and they found it in about 75 per cent (15 out of 23) of their "Group II" bloods. More recently Kline and his coworkers¹⁶ found this extra agglutinin, which they proved was identical with the agglutinin *c* of Guthrie and Huck, in 81 per cent of two hundred "Group II" individuals.¹⁷ So that once again, this time with respect to the agglutinin formula of "Group II" bloods, our general paralytics appear to present no difference from the usual

Following up the further work of Guthrie, Pessel and Huck,¹⁸ we then absorbed the serum of each "Group II" individual in our series with the corpuscles of a member of Group I, the absorbed serum was then cross agglutinated with the corpuscles of the same Group I individual and with those of five members of Group III. As is evident from Table IV, these various "Group II" sera did not behave alike, in that some of them, after absorption, continued to agglutinate Group III corpuscles as before, while others failed to do so.

TABLE IV

SERUM	ABSORBED WITH RBC	GR I (I K)	GR III (S J)	GR III (J H)	GR III (A F)	GR III (H B)	GR III (G K)
1 Group II (S M)	Group I (I K)	0	+	+	+	+	+
2 Group II (L D)	Group I (I K)	0	+	+	+	+	+
3 Group II (M H)	Group I (I K)	0	+	+	+	+	+
4 Group II (M I)	Group I (I K)	0	+	+	+	+	+
5 Group II (J H)	Group I (I K)	0	0	0	0	0	0
6 Group II (F G)	Group I (I K)	0	0	0	0	0	0
7 Group II (T K)	Group I (I K)	0	0	0	0	0	0
8 Group II (H R)	Group I (I K)	0	0	0	0	0	0

Since this Table IV is similar, on a reduced scale, to that published by Guthrie *et al*,¹⁸ we may quote their own words in this connection: "There was evidently an agglutinin (*D*) present in the serum of "Group II" [S M], "Group II" [L D], "Group II" [M H], and "Group II" [M I], which was not present in the serum of "Group II" [J H], "Group II" [F G], "Group II" [T K], and "Group II" [H R]. It is also plain that the corresponding agglutinin (*d*) was present in the erythrocytes of the five members of Group III, but absent from those of Group I." Now since each member of "Group II" agglutinated the corpuscles of all five Group III bloods, the two agglutinogens in the latter might have reacted either (1) with agglutinin *A* in patient J H and with *D* in patient S M, or (2) with *A* in patient J H and with *AD* in patient S M. But as Guthrie points out, each of the Group II sera agglutinated the cells of Group I, though it is evident that the latter are devoid of agglutinin *d* (Table IV), hence there must be some agglutinin other than *D* in all the "Group II" sera, therefore agglutinin *A* is present in all the "Group II" sera, and agglutinin *D* is present in addition in some of them."

We may schematize the foregoing as follows:

Table IV 1 AD- absorbed with -abe → D-
(gr II (S M) serum) (gr I (I K) cells) (absorbed serum)

D- cross agglutinated with -ad = +
(absorbed serum) (gr III cells)

Table IV	5	A -	absorbed with	-abc	→	O -
	(Gr II (J H) serum)		(Gr I (I K) cells)			(absorbed serum)
	O -	cross agglutinated with	-ad	= 0		
	(absorbed serum)		(Gr III cells)			

By combining the results of this experiment with those of the previous one, we find that the 'Group II' individuals in our series are represented by the agglutinin agglutimogen formulae above discussed in the following proportions

TABLE V

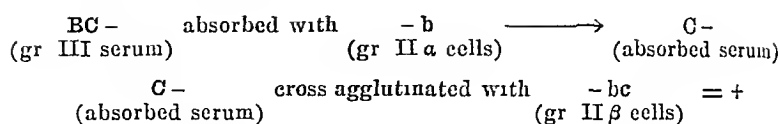
AD bc	27	} 22 containing D (86 per cent)
AD b	1	
Abc	2	} 2 not containing D
Ab	3	

Guthrie, Pessel and Huck¹⁸ state that they lack present information concerning the relative frequency of these types of 'Group II' bloods neither are we justified in drawing conclusions on this point on the basis of the few cases here reported, although it would seem that the presence of the extra agglutinin D and of the extra agglutimogen c is far commoner than the absence of either. On the other hand, these observers did not happen to encounter a type possessing neither the extra agglutinin nor the extra agglutimogen (ie formula Ab) though they speak of its probable existence. In the present series, however, we found three bloods (verified by repeated subsequent examinations) which lacked both agglutinin D and agglutimogen c. Our experience also differs from that of Guthrie to the extent that we were able to find but two examples of the absence of agglutinin D with the presence of agglutimogen c (formula Abc). The three types of Group II blood discovered and described by Guthrie and his co-workers¹⁸ occurred, then in our series of 37 'Group II' general paralytics in the proportions of 86 per cent (AD bc) 7 per cent (AD b) and 3 per cent (Abc) respectively the fourth possible type (Ab) which Guthrie did not happen to encounter made up 4 per cent of the group.

GROUP III CASES

The twelve Group III individuals in this series (one patient died before the further tests could be carried out) may be discussed very briefly.

It is clear that the two types of "Group II" blood which lack or possess agglutimogen c may be used to demonstrate the presence of agglutinins B and C. If a Group III serum be matched against a 'Group II' blood of the formula -b, agglutination will of course occur (Table I) the serum contains agglutinin B. If this agglutinin is now removed by absorption of the serum with the corpuscles of a "Group II" blood of formula -b and the absorbed serum then matched against the corpuscles of a 'Group II' blood of formula -bc the occurrence of agglutination must necessarily indicate the presence in the absorbed serum of agglutinin C.



Accordingly, we absorbed each of the twelve Group III sera in our series with the corpuscles of a "Group II" blood of known $-b$ formula, and then matched these absorbed sera, from which agglutinin B had thus been removed, against the corpuscles of a "Group II" blood of known $-bc$ formula. Strong agglutination took place in every instance, thus demonstrating the presence in each of the twelve Group III sera of agglutinin C as well as of agglutinin B (Table VI)

TABLE VI

SERUM	ABSORBED WITH RBC	SUBSEQUENT AGGLUTINATION OF LBC		
		GR II ($-b$) (JR)	GR II ($-b$) (JH)	GR II ($-bc$) (GH)
Group III (JH)	Group II (JH)	0	0	+
Group III (AR)	Group II (JH)	0	0	+
Group III (JS)	Group II (JH)	0	0	-
Group III (GK)	Group II (JH)	0	0	+
Group III (SJ)	Group II (JH)	0	0	+
Group III (HB)	Group II (JH)	0	0	+
Group III (DS)	Group II (JH)	0	0	+
Group III (LS)	Group II (JH)	0	0	-
Group III (BD)	Group II (JH)	0	0	-
Group III (DB)	Group II (JH)	0	0	+
Group III (CC)	Group II (JH)	0	0	+
Group III (VD)	Group II (JH)	0	0	+

It is equally clear that if the serum of a "Group II" blood of known formula $AD-$ be absorbed with the cells of a Group I blood (IK), agglutinin A will be removed from the "Group II" serum and agglutinin D remain, hence this absorbed serum, containing only agglutinin D , may be used to detect the presence or absence of agglutinin d . Accordingly, the serum of a "Group II" patient (GH, formula $AD-bc$) was absorbed with the corpuscles of a Group I patient (IK, formula $O-abc$), and the absorbed serum matched against the cells of each of the twelve Group III bloods in our series. Agglutination took place in every instance, thus demonstrating the presence in the corpuscles of each of the twelve Group III cases of agglutinin d , as well as of agglutinin a (since the same corpuscles are also agglutinated by "Group II" serum not containing agglutinin D).

This uniform agglutinin formula $-ad$ for the corpuscles of Group III individuals is in accord with the observations of Guthrie and Pessel,¹⁹ for they found agglutinin d associated with agglutinin a in the erythrocytes of seven members of this group. The agglutinin-agglutinin formula for the twelve Group III patients in this series thus appears to be $BCad$.

GROUP I CASES

It has already been seen that Group IV (JW) serum, after absorption with "Group II" ($-b$) corpuscles, was still able to agglutinate "Group II" ($-bc$) corpuscles, by virtue of possessing agglutinin C as well as agglutinin B .

If now from this absorbed serum agglutinin *A* is also removed by absorption with erythrocytes belonging to a Group III individual, this serum will contain only agglutinin *C*, and may therefore be used for the detection of agglutinin *c*. Accordingly the serum of J W, twice absorbed in this way, was matched against the corpuscles of the four Group I patients in our series. In each instance strong agglutination took place, demonstrating the presence in each of the four of agglutinin *c* as well as of agglutinogens *a* and *b* *.

The agglutinin agglutinin formula of all four of our Group I patients thus appears to be *O abc*. On the other hand, Guthrie and his coworkers^{13 10} found two Group I cases with the formula *O ab* and three with the formula *O abc*. It seems possible that the latter is the commoner.

THE WASSERMANN REACTION

Straszyński⁷ has recently demonstrated a certain degree of correlation between blood group and the readiness with which the Wassermann reaction disappears after antisyphilitic treatment. Having divided a series of 325 patients into 209 in whom the Wassermann became negative after only one or two courses of arsphenamine, and 116 whose Wassermann remained positive in spite of more prolonged treatment, he observed that to the latter category belonged 22 per cent of the 95 Group IV (Moss) individuals in the series, 36 per cent of the 119 Group II patients, 44 per cent of the 81 Group III patients, and 53 per cent of the 30 Group I patients. Our cases are hardly comparable to Straszyński's, not only because of being general paralytics, who are notoriously Wassermann fast but because the treatment which they received—malaria or tryparsamide or both—is scarcely calculated to bring about Wassermann negativity in the blood. Of the 71 patients in the present series observed for more than one year (some of them for periods up to three years in length) 70 per cent showed little or no change in the blood Wassermann, in 10 per cent the strength of the reaction had become definitely modified, in 20 per cent it had become essentially negative. When considered in relation to the blood group as shown in Table VII it is seen that the Wassermann reaction in the serum remained practically unchanged in 61 per

TABLE VII

WASSERMANN REACTION IN THE BLOOD	GROUP II NUMBER PER CENT	GROUP III NUMBER PER CENT	GROUP IV NUMBER PER CENT
Unchanged	17-61	10-91	21-75
++ to ++++ alc antigen			
+++ to ++++ chol antigen			
Definitely Modified	4-14	0	2-7
Neg to + alc antigen			
++ to ++++ chol antigen			
Negative	7-20	1-9	5-13
Neg alc antigen			
Neg to ++ chol antigen			
	28	11	28

* For Group I (11) corpuscles lack agglutinin *d* (Table IV) and absorption of Group III serum by Group I corpuscles leaves no agglutinin in the Group III serum for any of the four groups, both *B* and *C* being removed by the Group I corpuscles employed in the absorption.

cent of the 28 Group II patients, in 75 per cent of the 28 Group IV patients, and in 91 per cent of the 11 Group III patients, and became negative in 9 per cent of the Group III cases, in 18 per cent of the Group IV cases, and in 25 per cent of the Group II cases. No correlation could be discovered between the behavior of the Wassermann reaction and the actual agglutinin agglutinin formula of the "Group II" individuals.

Disregarding Group I, of which the members in our series are too few for inclusion, and making allowance for the small number of observations reported, our results are in accord with those of Straszynski (even though he found Group IV least Wassermann-fast, as compared with Group II in our material) at least to the extent that Groups II and IV, taken together, appear to present some contrast to Groups I and III with reference to the disappearance of the Wassermann reaction, but whether this suggests a correlation between the behavior of the Wassermann reaction in this respect and the presence of agglutinin A, since this is common to Groups II and IV and absent from both Groups I and III, it would naturally be hazardous to say. In any case, we hardly feel that our results offer anything in the way of comment upon those of Straszynski, indeed, considering the quantitative and qualitative limitations of our data, we are led to mention them only because it appears to us to be of some interest, in the light of his observations, that the few patients who exhibited any tendency to Wassermann-negativity under the nospirochetoidal type of therapy employed should nearly all have belonged to Groups II and IV, especially perhaps the former.

SUMMARY

1 The incidence of the traditional four isoagglutinin groups was found to be the same in a series of 91 patients with general paralysis as in the population at large. Since this is also true of syphilitics, general paralytics do not differ from syphilitics in general in this respect.

2 An extra agglutinin (*c*) in the corpuscles of "Group II" individuals, described by Guthrie and his coworkers and later by others, was found in the present series in the same proportion of "Group II" bloods (about 80 per cent) as reported by others.

3 An extra agglutinin (*D*) in the serum of "Group II" individuals, first described by Guthrie, was found in 32 out of 37 members of "Group II" in the present series (86 per cent).

4 Our patients, being general paralytics who received treatment only in the form of malaria or tryparsamide or both, showed little tendency to definite modification or negativity of the Wassermann reaction in the blood, all but two of the twenty patients who exhibited such a tendency were members either of Group II or Group IV (Moss), eleven of the latter eighteen belonging to Group II.*

*Since the foregoing article was submitted for publication there has appeared in the *Ztschr f d ges Neurol u Psychiat* of November 1926, a report of blood-group determinations carried out on a group of 100 general paralytics by Jacobsohn who found among these cases 3 per cent of group I, 47 per cent of group II, 17 per cent of group III, and 33 per cent of group IV individuals—essentially the same proportions as in our own series.

REFERENCES

- ¹Jansky J Hemtologische Studien bei Psychotikern, abstr in Jahresber f Neurol u Psychiat, 1907 u 1092
- ²Moss, W L Studies on Isoagglutinin and Isohemolysins, Tr Assn Am Phys, 1909, xxiv 419
- ³Hirschfeld L and H Scrologic Differences Between the Blood of Different Races, Lancet London, 1919, ii, 675
- Kilgore A R and Liu J H Isoagglutination Tests of Chinese Bloods for Transfusion Compatibility, Chin Med Jour, 1918 xxxii 21
- Moffitt G R, Klugh, G F, and Shepard, C E Blood Type Classifications with a Slight Modification of Technique Jour Am Med Assn 1919 lxviii 1821
- Liu, J H, and Wang, S H Isoagglutination Tests on One Thousand Chinese Bloods, Nat Med Jour China 1920 vi, 118
- Culpepper, W L and Ablson M Report of Five Thousand Bloods Typed, Using Moss' Grouping Jour Lab and Clin Med 1920 21 vi 276
- Cabrera C and Wade H W On the Isoagglutination Group Percentages of Filipino Bloods, Jour Philippino Islands Med Assn 1921 i 100
- Verzar, F, and Weszecky O Rassenbiologische Untersuchungen mittels Isohamagglutininen Biochem Zeitschr, 1921 22, cxlvi 33
- Tebbutt, A H, and McConnel S Human Isohemagglutinins Their Distribution Among Some Australian Aborigines Med Jour Australia 1922 i, 201
- Fukunishi H On the Biochemical Race Index of Koreans Manchus and Japanese Jour Immunol 1923 viii, 291
- Coen, A F and Dabert O A Study of the Occurrence of the Blood Groups Among the American Indians Jour Immunol 19_ viii 487
- Boi, W J and Verhoef A W On the Biochemical Index of Various Races in the East Indian Archipelago Jour Immunol, 1924 ix 53
- Armstrong, J S and Mathieson, F Blood Groups Among Samoans Brit Med Jour, 1924 ii 575
- Cleland, J B Blood Grouping of Australian Aborigines Australian Jour Exp Biol and Med Sc, 1926 iii, 33
- Snyder, L H Human Blood Groups Their Inheritance and Racial Significance Am Jour Phys Anthropol 1926 iv, 233 The latter named contains a tabulation of the various racial distributions found by a large number of workers, including most of those here cited and numerous other)
- Furuhata T, and Kishi T On the Biochemical Racial Index of the Japanese in the Hokuriku District (northern part of middle Japan) Jour Immunol 1926, xii 83
- Kishi, T On the Biochemical Racial Index of the Ainu Gilyak and Orochko in Karo futo (Saghalien) Jour Immunol 1926 xii 91
- ⁴Verzar and Weszecky
- ⁵Ottenberg, R, and Epstein L Trans N Y Path Soc 1908 vii 117
- Von Dungern, F, and Hirschfeld D L Ueber Vererbung gruppenpezifischer Strukturen des Blutes Zeitschr f Immunitat u Infekt 1910 vi 254
- Otteuberg R Medicolegal Application of Human Blood Grouping Third Communication Jour Am Med Assn 1922 lxxv 2137
- Otteuberg R Hereditary Blood Qualities Statistical Considerations Jour Immunol, 1923, viii, 11
- Buchanan J A A Consideration of the Various Laws of Heredity and Their Application to Conditions in Man Am Jour Med Sc 19_3, cxlv 675
- ⁶Bernstein F Zusammenfassende Betrachtungen uher die erblichen Blutstrukturen des Menschen Ztschr f ind Abst u Vererb 192_ xxxvii 237
- ⁷Straszynski, A Ueber das Ergebnis der Wassermannschen Reaktion innerhalb verschiedener Blutgruppen bei behandelter Lues Klin Wchnschr, 192_ iv 1962
- ⁸Hirschfeld, H, and L and Brokman H On the Susceptibility to Diphtheria (Schick test positive) with Reference to the Inheritance of Blood Groups Jour Immunol, 1924 ix 571
- ⁹Weitzner G Hamagglutinationsgehalt des Bluteserums Karzinomkranker Med Klin 192_ xxi 1960
- ¹Bendin S G T Hamagglutinationsgehalte van het bloedserum bij carcinoompatienten Nederl. Tijd. v Geneesk 1926 lxx 2856
- Alexander W An Inquiry into the Distribution of the Blood Groups in Patients Suffering from 'Malignant Disease Brit Jour Exp Path 19_1, ii 66
- Buchanan J A and Hely E T The Relation of Blood Groups to Disease Brit Jour Exper Path, 19_1 ii 247
- Pfahler G E and Widmann B P The Relation of Blood Groups to Malignant Disease and the Influence of Radiotherapy Am Jour Roentgenol 1924 vii, 47

- ¹¹Landsteiner, K Ueber Agglutinationsercheinungen normalen menschlichen Blutes, Wien klin Wchnschr, 1901, *xiv*, 1132
- ¹²Guthrie, C G, and Pessel, J F Further Studies on Blood Grouping II The Influence of Temperature upon Isohemagglutination, Bull Johns Hopkins Hosp, 1924, *xxv*, 33
- Jevrell, F The Influence of Temperature upon the Agglutination of the Red Blood Corpuscles Jour Immunol, 1921, *vi*, 445 (Cited by Guthrie and Pessel)
- ¹³Guthrie, C G, and Huck, J G On the Existence of More Than Four Isoagglutinin Groups in Human Blood, Bull Johns Hopkins Hosp, 1923, *xxiv*, 37, 80, 128
- ¹⁴Huck, J G, and Guthrie, C G Further Studies on Blood Grouping I The Antigenic Properties of Two Types of "Group II" Erythrocytes, Bull Johns Hopkins Hosp, 1924, *xxv*, 23
- ¹⁵Coea, A F, and Klein, H A Hitherto Undescribed Pair of Isoagglutination Elements in Human Beings Jour Immunol, 1923, *viii*, 477
- ¹⁶Kline, B S, Ecker, E E, and Young, A M The Incidence of Two Types of Group II Human Red Cells Jour Immunol, 1925, *v*, 595
- ¹⁷Vorsehutz, J Worauf beruht das Wesen der einfachen wie der Gruppenagglutination und die verschiedene Ladung der roten Blutkorperehen, Ztschr f klin Med, 1923, *xcvi*, 383
- Landsteiner, K, and Witt, D H Observations on Human Isoagglutinins, Proc Soc Exp Biol and Med, 1923 *24*, *xi*, 389
- Tebbutt, A H Irregularities in Isoagglutination Med Jour Australia (Supplement), 1924, *1*, 234 (cited by Snyder)
- Mino, P Ueber die angebliche Existenz von mehr als zwei Isoagglutininen im menschlichen Blute, Munchen med Wchnschr, 1924, *lxii*, 1129
- Lattes, L, and Cavazzuti, A Sur l'existence d'un troisième élément d'isoagglutination, Jour Immunol, 1924, *ix*, 407
- Landsteiner, K, and Witt, D H Observations on the Human Blood Groups, Irregular Reactions Isoagglutinins in Sera of Group IV The Factor A Jour Immunol, 1926, *xi*, 221
- Ottenberg, R, and Johnson, A A Hitherto Undescribed Anomaly in Blood Groups, Jour Immunol, 1926, *xii*, 35
- ¹⁸Guthrie, C G, Pessel, J F, and Huck, J G Further Studies on Blood Grouping V The Recognition of Three Types of "Group II" Blood, Bull Johns Hopkins Hosp, 1924, *xxv*, 221
- ¹⁹Guthrie C G, and Pessel J F Further Studies on Blood Grouping IV The Demonstration of two additional Isoagglutinins (D and Q) in Human Blood Bull, Johns Hopkins Hosp, 1924, *xxv*, 126

MESENTERIC ENTEROCYSTOMA*

By WILHELM HUEPER, M D, CHICAGO, ILL

CYSTIC tumors in the mesentery are relatively rare. The majority of cystic formations located in the mesentery are not genuine neoplasms but are cysts of various origins as parasitic or bacterial infection (ecchinococcus cysts, cysts of the cysticercus cellulosae gas cysts known as pneumatosis cystoides intestini) or due to trauma of the abdomen causing a localized hemorrhage or lymphorrhagia, central necrosis and liquefaction of infected lymph glands (tuberculosis typhoid fever) or of solid tumors (lipomas). Among the true tumors of the mesentery the cystic new growths exceed by far in number the solid neoplasms (ratio 4:1 according to Martin¹). After being formerly classified according to their content (blood, lymph, chylous, caseous material) the mesenteric cysts are grouped at present according to their origin and histologic structure because the content is not a sufficient criterion of the actual character of the tumor.

Classification

- 1 Cystic lymphangiomas
 - a Serous cysts
 - b Chylous cysts
- 2 Enterocystomas
- 3 Cysts being derived from the wolffian duct
- 4 Dermoid cysts
- 5 Teratomas
- 6 Fetal inclusions
- 7 Teratoid mixed tumors

FREQUENCY

The cystic lymphangiomas rank highest in number with more than 200 cases reported (Forster²). The frequency in the other groups is much lower: enterocystomas 27, cysts being derived from the wolffian duct 5, dermoid cysts 33, teratomas 8, fetal inclusions 9 and teratoid mixed tumors 2 (Dowd,³ Niosi,⁴ Colmers,⁵ Sommer⁶).

On account of the rarity of the mesenteric enterocystomas and with reference to the pending discussion of their origin we wish to report a new case of mesenteric enterocystoma which came recently to our observation.

REPORT OF CASE

HISTORY—B. S., boy aged 5 was admitted to Mercy Hospital in May, 1925 previous history negative, except tuberculous lymph glands on the neck in the last two years. Three days prior to admission, the patient became suddenly sick with pains in the stomach region.

III From the Department of Pathology Loyola University School of Medicine Chicago.
Received for publication August 6 1926.

lasting until the next morning. The patient was feeling well till in the evening the pains started again with increased strength, but localized more around the navel and combined with vomiting during the night. The pains subsided again in the morning of the third day having a new onset in the afternoon, when the child was first seen by Dr I. F. Golden, to whom I am greatly indebted for the clinical history of the case. The abdomen was slightly distended at that time, the right hypogastric region was tender and showed muscular rigidity. A hen egg sized, movable tumor of an elastic consistency and of a smooth surface was felt in the right hypogastric region near the navel. Leucocyte count 22,400. *Diagnosis:* Acute appendicitis and mesenteric cyst. A hen egg sized cyst located between the two membranes of the mesentery, $2\frac{1}{2}$ inches from the ileocecal valve adjacent, but not connected with the ileum was enucleated at operation. Also appendectomy was performed.

Histologic examination—The wall of the cyst was about 0.2 cm. thick, showing on the rather smooth inner side a small nodule, rice seed size, projecting into the lumen. Microscopically the wall was composed of five layers: serosa, subserosa, muscularis, submucosa and mucosa. Serosa and muscularis showed a similar structure to that found in the corresponding layers of the normal intestinal wall. The submucosa was a rather dense connective tissue and thinner than the same layer in the normal intestinal wall. The mucosa was formed by a single layer of cubical or low cylindrical epithelial cells. Goblet cells were not observed. Rudimentary villi were present in a small area. Glandular formations, resembling in location and structure Brunner's glands were seen in some places. In other parts of the wall the epithelial lining was completely lacking. Lymphoid tissue was only found in the above-mentioned nodule. The appendix showed a moderate hyperplasia of the lymph follicles. There were no signs of an acute inflammation.

DISCUSSION

The mucosa and submucosa of the cyst just described represent the type of intestinal structure which is present in an early stage of fetal life. At that time the intestinal mucosa is composed of a single layer of cylindrical cells and does not show any villi. Also the lymphoid tissue in the submucosa is not yet developed in this stage of prenatal development. In other cases a complete imitation of a fully developed intestinal mucosa with villi and glandulae Lieberkuhn was reported. But irregularities and defects in the development of individual layers were frequently seen, as lack of the lymphoid tissue or of one layer of the muscularis or as partial or complete fibrosis of the muscularis. Structural imperfections of the last-mentioned kind will naturally enhance greatly the difficulty of an exact interpretation. But in discarding those tumors from this group only the complete lack of muscular tissue may justify such a step. Investigations of Schmitt⁷ have shown that sometimes very small rests of muscular tissue may be present in an entirely fibrotic middle layer.

ORIGIN

The majority of the enterocystomas originate from rests of the omphalo-mesenteric duct. The most common malformation resulting from an incomplete obliteration of this duct on its intestinal end is the Meckel's diverticulum, which is found in 2 per cent of men. In other cases the duct remains open through its whole length or only in its distal or central part. Rests of this kind may gain a certain autonomy and cystic tumors of the structure and character of enterocystomas are the result. They are found in accordance with the location of the omphalomesenteric duct, in the abdominal wall near

the navel, in the mesentery, and on the convex side of the intestine (Colmeis⁵) From 43 enterocystomas 4 were in the abdominal wall, 25 on the convexity of the intestine and 15 on the concave side of the intestine or in the mesentery. Corresponding with the usual location of Meckel's diverticulum 80 cm. above the ileocecal valve on the ileum the enterocystomas are seen most frequently in this region of the ileum or in the adjacent mesentery. But the great variation in the location of the diverticulum which is found on the small intestine from the duodenum to the ileocecal valve explains also the occurrence of enterocystomas along this line either connected with the bowel or free in the mesentery.

But evidently not all enterocystomas are derived from rests of the omphalomesenteric duct. There are 2 cases reported in which multiple cysts were observed. Roth⁸ described the occurrence of many small cysts along the ileum in the mesentery and Schminke⁹ saw one cyst in the mesentery and a second one in the dorsal mediastinum. The peculiar location of the multiple cysts in these two cases is an absolute argument against their origin from rests of the omphalomesenteric duct. The source of these cysts was presumably displaced embryonal intestinal tissue. Observations of Elze¹⁰ on the fetal intestine where he found epithelial formations interpreted by Aschoff¹¹ as processes of epithelial cells into the intestinal wall may offer some substantiation of this conception if we consider that these processes may become displaced to the outside of the intestine.

Also during my own investigations on the duodenum of thirty rats and rabbits I found in the wall of the duodenum of one rabbit a small cyst located in the subserosa. The cyst had a well developed muscularis and a mucosa which was similar to that of the duodenum only thinner. We are more inclined to interpret this cyst as a malformation of the intestinal wall on account of its close relation to it than as a rest of the omphalomesenteric duct. If the negative results of Wojciechowski who examined without success the mesentery of 48 human bodies for displaced intestinal tissue seem to disagree with our conception, we have to consider the fact that the small number of cases examined does not allow any definite conclusions in this direction.¹² Furthermore we do not believe that enterocystomas may be produced by the traction of displaced pancreatic tissue on the intestinal wall causing diverticula (Hansson¹³), because in none of the cases reported was pancreatic tissue found in the wall of the cysts.

The enterocystomas are generally of minor size. They rarely exceed the size of an apple although larger ones were observed. Usually there is only one cyst. Rarely two are present connected with each other by a duct. Connections of the lumen of the cyst with the lumen of the intestine through a duct have been described especially in cases which the cyst was adherent to the wall of the intestine. Malignant degeneration in the epithelial part of the cyst was found in one case (Schmitt¹⁴) in which a carcinomatous growth was present in nodular formations projecting into the cystic lumen.

CONCLUSIONS

- 1 A case of mesenteric enterocystoma is reported
- 2 Enterocystomas may originate from rests of the omphalomesenteric duct or from displaced embryonal intestinal tissue

REFERENCES

- ¹Martin, A. *Deutsche Chirurgie*, 1903, Enke, Stuttgart
- ²Forster, E. *Bruns' Beitr z klin Chirurgie*, 1921, cxvii, 116
- ³Dowd. *Ann of Surg*, 1900, xxxii, 515
- ⁴Niosi. *Virchow's Archiv f path Anat*, 1907, cxc, 217
- ⁵Colmers. *Arch f klin Chirurgie*, 1906, lxxix, 132
- ⁶Sommer, R. *Bruns' Beitr z klin Chirurgie*, 1921, cxix, 84
- ⁷Schmitt. *Zentralbl f Chirurgie*, 1923, I, 1723
- ⁸Roth. *Virchow's Arch f path Anat*, 1881, lxxxi, 371
- ⁹Schmuncke. *Virchow's Arch f path Anat*, 1920, ccxxvii, 12
- ¹⁰Elze. *Inaug Diss*, Freiburg, 1909
- ¹¹Aschoff. *Pathologische Anatomie*, ed 4, 1919, p 858
- ¹²Wojciechowski. *Polska Gaz Lekarska*, 1922, No 42 (cit *Zentralbl f Chirurgie*, 1924, li, 573)
- ¹³Hansson. *Hygiea*, 1917, lxxix, 1122

THE USE OF CHOLIN IN PARALYTIC ILEUS*

BY HANS HARTMAN, M D, AND WM DOCK, M D, SAN FRANCISCO, CALIF

IN 1925 Klee and Grossman¹ reported encouraging results from the administration of cholin intravenously in 120 cases of paralytic ileus. At the same time Magnus² summarized the experimental work which had been done in his laboratory on cholin in its relation to the gastrointestinal tract. Cholin is normally present in the muscularis of the stomach, and the large and small bowels. It diffuses out of fresh strips of gut and the fluid thus obtained reestablishes contraction in old intestinal strips from which the cholin has disappeared. While cholin is not absent in the gut in cases of ileus, the intravenous administration causes a return of normal peristalsis in experimental ileus due to infection, prolonged anesthesia, or trauma. The single lethal dose in animals was found to be 35 mg per kilogram. Over a period of one hour 50 mg per kilogram could be given with safety. The dose suggested for man was 600 mg per 60 kilograms given slowly (seventeen minutes). Cholin (trimethyloxyethyl ammonium hydroxide) is given as the hydrochloride, and the crystals or solution should be kept in ampules to prevent decomposition to more toxic substances.

Wolf and Canney³ in 1926 reported a series of four cases in three of which cholin was successfully used, in the fourth the administration had been too long delayed and there was no effect. Six hundred mg diluted in 180 cc of sterile normal saline solution was given intravenously over a period of time not shorter than seventeen minutes.

*From the Departments of Surgery and Medicine Stanford University Medical School San Francisco Calif

Received for publication July 25 1926

As we were unable at the time to obtain cholin from the firm which supplied the above workers we accordingly felt it necessary to standardize the American product (Eastman Kodak Company) as to toxicity. Rabbits were used for this purpose and it was found that 50 mg per kilogram in a single dose was well tolerated. The lethal dose was 75 mg per kilogram given in a single dose. The therapeutic dose produced marked salivation, redness of the ears, contraction of the pupils, slowing of the pulse and peristaltic action even in anesthetized animals in which the gut had been traumatized until ileus resulted.

We have had an opportunity to use this drug in but a single case of ileus but the immediate result was so striking that further trial seems justified.

An obese woman, forty years old developed abdominal distention and vomiting following hysterectomy. Gastric lavage, stipes and enemas given over a period of four days failed to relieve the condition. She had no bowel movements or passage of gas. Her temperature rose to 103 and vomiting of fecal material occurred. No peristaltic sounds could be heard. The diagnosis was paralytic ileus possibly with peritonitis. She was given 0.6 gm of cholin hydrochloride in 250 cc of normal saline solution over a period of twenty minutes. The blood pressure fell from 120 to 80 the first three minutes and there was flushing of the face, salivation but no appreciable slowing of the pulse rate. Immediately after the intravenous administration had been completed peristalsis was heard and the patient expressed a desire to defecate and in about five minutes passed a large quantity of gas and liquid feces. There were no cramps. The temperature continued to rise and the patient died in twelve hours. At necropsy a partial obstruction without gangrene was found high in the jejunum which had herniated into the abdominal wound.

CONCLUSION

The use of cholin intravenously in paralytic ileus is justified on both a clinical and experimental basis. The action both therapeutic and toxic is very fleeting, the rate of administration should be controlled by following the blood pressure. One half to 1 gm can be given in fifteen to thirty minutes and repeated if necessary at two to three hours intervals. The cholin should be kept in sealed ampules containing the approximate dose either in crystals or solution as the decomposition products are quite toxic.

REFERENCES

- ¹Klee and Grossman. Ueber die klinische Brauchbarkeit des Cholins. München med. Wehnschr. 1925, lxxii 251-254.
- ²Magnus. Die physiologische und therapeutische Bedeutung des Cholins für die Magendarmtätigkeit, München. med. Wehnschr. 1925 lxxii 249-251.
- ³Wolf and Canney. The Treatment of Ileus by Cholin. Lancet 1926 i 707-708.

SUGAR IN BLOOD*

A STUDY OF THE ACCURACY OF THE KRAMER-GITTLEMAN MODIFICATION OF THE METHOD OF FOLIN AND WU

By ELSA R. ORENT, B.S., BROOKLYN, N. Y.

A SURVEY of the literature reveals a surprisingly large number of methods for the estimation of sugar in blood. Most of these require quantities of blood such as can only be obtained by entering a vein. A few,¹ of which the Bang² method is probably the most widely used, require such quantities of blood as can be obtained by pricking the finger. Such a method has recently been described by Kramer and Gittleman.³ In their technique, the blood is collected in a small pipette, $\frac{1}{20}$ to $\frac{1}{10}$ of a cc of blood being required for a single determination.

The principle of the method is the same as that used by Folin and Wu.⁴ The advantage of using minute quantities of blood, obtained without venipuncture, is obvious, especially in work on infants and small animals. However, the accuracy of the microtechnique has been questioned in some quarters. I have, therefore, undertaken an intensive study of this method in comparison with the one originally described by Folin and Wu. This paper gives the results of this study.

PROCEDURE

One cc of distilled water is put into a small tube graduated at 2 cc. The finger from which the blood is to be obtained is then washed with alcohol and ether, and pricked with a lancet. One-twentieth or $\frac{1}{10}$ cc of blood is drawn into a pipette, graduated at 0.05 cc and 0.1 cc. Where the blood-sugar concentration is expected to be high only $\frac{1}{20}$ cc of blood is required. The blood is transferred from the pipette into the tube containing the distilled water. Great care must be taken in measuring the blood. After transferring the blood into the tube, the pipette must be well washed by drawing the water up into the tube several times. The tube is rolled between the palms of the hands in order to produce complete hemolysis. One-tenth cc of 10 per cent sodium tungstate is added, followed, after mixing, by 0.1 cc of $\frac{2}{3}$ N sulphuric acid to precipitate the proteins. The tube is again rolled until the mixture turns dark brown. The volume is then made up to the 2 cc mark and the tube again rolled. The tube is allowed to stand five minutes, then centrifuged about five minutes, and the supernatant fluid aspirated and transferred to a 10 cc graduated cylinder. Care must be taken not to disturb the protein precipitate. Using a pipette graduated in $\frac{1}{10}$ cc an aliquot (1.5 cc was used here) of the supernatant fluid is meas-

*From the Harry Caplin Pediatric Research Laboratory and the Pediatric Department of the Jewish Hospital of Brooklyn, N. Y.
Received for publication July 25, 1926.

TABLE I
DETERMINATION OF SUGAR IN SOLUTIONS CONTAINING KNOWN AMOUNTS OF GLUCOSE

AMT OF SUGAR ACTUALLY PRESENT IN 100 C.C. SOL.	50 MG		100 MG		150 MG		200 MG		250 MG		300 MG		350 MG	
	K-O	F-W	K-O	F-W	K-O	F-W	K-O	F-W	K-O	F-W	K-O	F-W	K-O	F-W
	49.8	49.3	102.7	99.0	149.3	149.4	200.3	202.6	249.1	251.5	299.0	298.5	350.5	353.8
	51.0	49.0	100.7	99.0	165.0	150.9	200.9	200.9	251.4	251.0	302.9	303.0	353.0	350.9
	48.7	50.0	100.0	95.8	151.5	151.2	200.5	195.4	249.7	253.1	301.9	299.3	350.6	347.8
	48.4	46.1	100.0	98.1	150.3	149.8	203.0	191.0	249.2	253.1	300.4	298.5	352.0	347.8
	49.0	52.4	102.8	101.0	151.3	151.5	203.2	203.2	249.9	253.1	300.9	303.0	350.5	353.8
	49.7	50.9	102.9	100.5	149.9	149.3	201.3	202.0	249.0	253.1	300.8	298.5	350.0	350.9
	50.7		104.1		152.4		200.4		250.8		303.0		349.7	
	49.3		99.4		146.9		0.4		251.8		300.6		351.8	
	50.7		101.1		150.1		201.6		250.1		300.6		350.9	
	49.9	49.7	101.1	98.9	150.3	150.3	201.6	200.1	250.1	252.2	300.6	299.6	350.9	350.8
	0.1	1.5	1.4	1.3	1.8	0.2	1.7	2.7	1.1	2.7	1.4	3.4	4.2	5.3
	1.2%	2.0%	1.4%	1.0%	1.5%	0.1%	0.8%	1.3%	0.4%	1.1%	0.5%	0.8%	0.6%	0.7%
	0.9	1.9	2.0	2.0	2.3	0.9	2.3	2.9	1.1	2.9	1.6	2.5	1.5	2.6

These solutions were made up by Dr Murray J. Shear. The author was given the values of the concentrations of these known solutions after the analyses were completed.

†K-O = Folin and Wu

†F-W = Folin and Wu

In calculating the standard deviation the amount of glucose actually present in 100 c.c. solution was used as the mean

TABLE II
SUGAR CONCENTRATION IN BLOOD OF PATIENTS

KRAMER GITTLEMAN		FOLIN WU		
MG PER 100 CC OF BLOOD	DIFFERENCE IN % BETWEEN CHECK DETERMINATIONS	MG PER 100 CC OF BLOOD	DIFFERENCE IN % BETWEEN CHECK DETERMINATIONS	DIFFERENCE IN % BETWEEN THE MEANS OF THE TWO METHODS
A NONDIABETIC				
85.7		85.8		
102.5		104.4		
103.7	1.2%	105.6	1.1%	1.8%
104.1		108.0		
103.9	0.2%	106.4	1.5%	3.0%
104.7		103.0		
102.9	1.7%	101.4	1.6%	1.5%
81.5		84.4		
82.9	1.7%	84.4	0.0%	2.6%
99.9		99.8		
98.7	1.2%	96.9	2.9%	1.0%
91.7		94.5		
92.4	0.8%	93.7	0.9%	2.2%
87.1		86.0		
85.6	1.7%	85.2	0.9%	0.8%
77.8		79.8		
75.0	0.3%	78.1	1.7%	1.5%
76.9		77.3		
78.1	1.5%	79.2	2.0%	1.6%
102.0		100.0		
103.0	1.0%	101.0	1.0%	1.9%
Δ	1.1%	Δ	1.3%	Δ 1.8%
B DIABETIC				
400.0		400.0		
396.7	0.8%	400.0	0.0%	0.4%
253.8		256.4		
256.4	1.0%	259.7	1.2%	0.4%
280.1		277.8		
278.5	0.6%	276.7	0.4%	0.7%
209.7		207.0		
208.9	0.4%	206.1	0.4%	1.1%
216.5		216.4		
216.0	0.2%	216.4	0.0%	0.2%
Δ	0.6%	Δ	0.4%	Δ 0.6%

ured into a modified Folin-Wu sugar tube graduated at 4 cc and 6 cc. Two standards are prepared using 1 cc and 0.5 cc respectively of a solution containing 0.1 mg of sugar. Two cc of the alkaline copper solution are added to each tube and the volume is made up to 4 cc. The tubes are heated in a boiling water-bath for six minutes, transferred to a cold water bath and allowed to stand three minutes. If the tubes are heated longer than six minutes, reoxidation takes place, and low results are obtained. Two cc of the phosphomolybdate solution are added to each tube and the volume is made up to 6 cc. After letting the tubes stand from five to ten minutes to permit the escape of CO₂ gas, the solutions are ready to be read in

TABLE III
SUGAR CONCENTRATION IN BLOOD OF DOGS
IN MG PER 100 CC OF BLOOD

DOG 52		DOG 53		DOG 54		DOG 57		DOG 61	
K-G	F-W	K-G	F-W	K-G	F-W	K-G	F-W	K-G	F-W
102 0	109 0	81 2	78 4	87 1	88 1	87 3	86 0	94 9	94 0
106 4	109 0	78 8	78 4	90 1	87 4	85 4	87 5	93 5	91 5
128 7	129 0	124 2	123 0	100 0	98 4	81 3	83 4	92 4	81 3
126 5	129 0	123 8	128 6	101 2	98 4	82 08	83 4	83 3	82 0
		110 5	112 3					87 2	80 0
191 3†	192 3	107 1	113 0	103 0	101 6	87 1	87 0	88 2	87 1
192 9	194 1			103 0	105 0	87 1	86 0	88 0	86 0
161 3	163 9	112 8	115 0	102 4	104 2	85 7	81 05	86 2	86 0
164 7	165 2	115 2	114 2	104 0	105 2	83 9	82 0	73 0	71 6
144 9	147 0	150 9†	150 0	104 0	105 0	81 8	83 4	75 7	73 2
146 3	147 0	150 9	154 2	103 0	105 0	80 3	82 6	71 1	72 5
137 6	138 2	153 0	158 0	106 0	105 7	74 1	73 2	72 1	72 5
135 2	137 9	150 9	154 2	106 0	104 2	71 9	74 0	39 1‡	38 5
127 1	129 0	148 7	150 0	74 0	74 5	22 7‡	20 0	35 0	33 0
125 3	129 0	146 7	150 0	33 3‡	34 0	24 3	20 7		
101 3	99 5	108 1	107 0	31 0	32 8				
		119 6	121 2						
125 0	122 6	121 3	121 5						
125 0	124 2	122 6	121 9						
		119 6	121 2						

These blood sugar determinations were made in connection with the study of the effect of ligation of the hepatic artery on carbohydrate metabolism

†Blood sugar determinations after injection of adrenalin

‡Blood sugar determinations following hypoglycemic shock

the colorimeter. The standard is set at 15 and read against itself. If the colorimeter is properly adjusted the two standards should give the same reading. One standard is then replaced by an unknown and several readings taken. While the solutions are being compared in the colorimeter, care should be taken that no gas bubbles collect about the prisms.

Calculations—

$$\frac{\text{Reading of standard} \times \text{mg of glucose in standard} \times 100}{\text{Reading of unknown} \times \text{amount of blood present in the aliquot}} = \text{mg of sugar per 100 cc of blood}$$

e.g.

When 0.1 cc. of blood is used 15 cc. of supernatant fluid represents 0.075 cc. of blood. If this gives a reading of 20 against a 0.1 mg. standard set at 15 then the calculation is as follows:

$$\frac{15 \times 0.1 \times 100}{20 \times 0.075} = 100.0 \text{ mg of sugar per 100 cc. of blood}$$

or to simplify the calculation divide 2000 by the reading

$$\frac{2000}{\text{Reading}} = \text{mg sugar in 100 cc of blood}$$

$$\frac{2000}{20} = 100 \text{ mg of sugar per 100 cc of blood}$$

RESULTS

Table I gives the results of a series of comparative determinations by the original Folin Wu method and by the Kramer-Gittleman modification on solutions of known concentrations ranging from 50 mg to 350 mg per 100 cc.

The average amount of glucose recovered in each solution is shown. The mean error has been determined in terms of milligrams per 100 cc and per cent. Using the amount of glucose known to be present as the mean, the standard deviation from this quantity has been calculated.

Table II, first and third columns, gives the results of comparative determinations by the Folin-Wu method and by the Kramer-Gittleman method on the blood of a series of nondiabetic and diabetic patients. The difference in per cent between check determinations with each method is given in the second and fourth columns respectively. The difference in per cent between the average values obtained with these two methods is given in the last column.

Table III shows a series of comparative determinations made on the blood of dogs. These dogs were used in a study of the relation of the blood sugar level to the convulsive seizure following ligation of the hepatic artery.

CONCLUSIONS

1 The average reproducibility of the Kramer-Gittleman method is about 10 per cent, that of the Folin-Wu is also about 10 per cent.

2 The values obtained by the Kramer-Gittleman technique agree with those obtained by the Folin-Wu technique to within 20 per cent. This is only slightly greater than the degree of reproducibility of either method.

3 These findings show that the microtechnique of Kramer and Gittleman for the quantitative determination of substances in blood that reduce alkaline cupric hydroxide solutions gives results that compare well with those obtained with the original method of Folin and Wu.

The author is indebted to Dr Benjamin Kramer for his advice and encouragement in this investigation.

REFERENCES

- 1 Epstein, A. A. Jour Am Med Assn, 1914, lvi, 1667
- 2 Fitt, R. Arch Int Med, 1914, xiv, 133
- 3 Kahn, M. Jour Am Med Assn, 1915, lvi, 241
- 4 Hopkins, A. H. Jour Am Med Assn, 1915, lvi, 254
- 5 Lewis, R. C. and Benedict, S. R. Jour Biol Chem, 1915, xx, 61
- 6 Macleod, J. J. R. Jour Lab and Clin Med, 1915-1916, i, 645
- 7 Hagedorn, H. C. and Jensen, N. B. Ugeskr f Laeger, 1918, lxxx, 1217
- 8 Maclean, H. Biochem Jour, 1919, xiii, 135
- 9 Wallis, R. L. M. and Gallagher, C. D. Lancet, 1920, ii, 781
- 10 Host, H. F. and Hattchell, R. Jour Biol Chem, 1920, xli, 347
- 11 Kleiner, I. S. Jour Am Med Assn, 1921, lxxvi, 172
- 12 Thalhimer, W. and Updegraff, H. Jour Am Med Assn, 1922, lxxviii, 1583
- 13 Pollock, H. O. and McElroy, W. S. Am Jour Med Soc, 1922, cxliii, 371
- 14 Braumann, E. J. and Ingerson, R. L. Jour Lab and Clin Med, 1922, i, 357
- 15 Culvert, E. G. B. Biochem Jour, 1923, xvii, 117
- 16 Randles, F. S. and Grigg, W. K. Jour Am Med Assn, 1924, lxxxviii, 684
- 17 Kiefer, E. D. Jour Am Med Assn, 1925, lxxxix, 569
- 18 Byrd, T. L. Jour Lab and Clin Med, 1925, ii, 67
- 19 Gilbert, M. and Bock, J. C. Jour Biol Chem, 1924-1925, lxxv, 61
- 20 Bang, L. Biochem Zeitschr, 1913, lvi, 300
- 21 Kramer, B. and Gittleman, I. F. Jour Am Med Assn, 1923, lxxx, 1171
- 22 Folin, O. and Wu, H. Jour Biol Chem, 1920, xli, 367
- 23 Collens, Wm. S., Shelling, D. H., and Byron, Chas. S. Proc Soc Exp Biol and Med, 1926, xxi, 361, Am Jour Physiol, 1926, lxxxviii, 349
- 24 Idem. Proc Soc Exp Biol and Med, 1926, xxi, 515

NORMAL BLOOD COUNTS IN PIGEONS*

By FROID DE LBS PH D SAN FRANCISCO, CALIF

DURING the course of studies on the anaphylactoid and anaphylactic shock reactions in pigeons¹ in this laboratory a large amount of data on normal blood counts has been accumulated. In view of the limited information on this subject available in the literature it seemed worth while to place this data on record.

I am familiar with only two reports on pigeon blood in the literature. Klieneberger and Carl made a study of the blood morphology of six pigeons. They reported the following counts: erythrocytes from 3,780,000 to 4,535,000; leucocytes from 10,430 to 31,430; and thrombocytes (platelets) from 9,070 to 63,490 per cu mm. The differential leucocyte count also showed great variability. A similar variability in cell counts has been reported by Arlong and Dufourt² but they do not state the number of pigeons used.

The blood counts reported in this paper were made on normal pigeons using blood from two regions, heart blood i.e. blood from the heart by chest puncture, which represented the main channel of the circulation and blood from a superficial vein of the leg which represented a peripheral channel. For differential staining Hissings³ modification of the Nocht stain

TABLE I
NORMAL BLOOD COUNTS IN PIGEONS

TYPE OF CELL	HEART		LEFT VEIN	
	RANGE	MEDIAN	RANGE	MEDIAN
Small lymphocytes	5 to 53%	30% (21)	2 to 13%	30% (33)*
Large lymphocytes	9 to 67%	21% (23)	3 to 61%	21% (33)
Pseudo eosinophilic polymorphonuclears	0 to 25%	3% (20)	0 to 31%	1% (33)
Eosinophilic polymorphonuclears	2 to 75%	20% (23)	14 to 50%	27% (33)
Eosinophilic myelocytes	0 to 21%	0% (23)	0 to 4%	0% (33)
Basophilic myelocytes	0 to 3%	0% (23)	0 to 2%	0% (30)
Basophilic polymorphonuclears	0 to 2%	0% (23)	0 to 7%	0% (33)
Leucocytes	7,600 to 16,600	11,500 (6)	3,600 to 8,600	5,200 (6)
Erythrocytes	2,625,000 to 4,325,000	3,300,000 (25)	2,275,000 to 4,475,000	3,300,000 (26)
Thrombocytes	8,000 to 89,000	24,000 (32)	8,000 to 84,000	38,000 (45)

*Number of pigeons used in parentheses

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.

Received for publication July 22, 1946

was employed. For counting, the diluting fluid of Rees and Ecker³ was used for erythrocytes, thrombocytes and leucocytes, the erythrocytes remained unstained and all other cells were stained blue. The results obtained are presented in the accompanying table.

The results confirm the great variability in blood counts of pigeons previously reported by others. The differential counts on leucocytes were originally made with the view of correlating certain changes in these cells with the anaphylactoid and anaphylactic shock reactions just as had been done with the erythrocytes and thrombocytes in a previous study,¹ but the counts were too variable to permit the drawing of definite conclusions. This was true of the leucocyte counts in the experiments individually and collectively. In the previous study,¹ the counts of thrombocytes and erythrocytes relative to each other were more uniform and permitted definite conclusions. It is obvious that caution should be exercised in drawing conclusions from blood counts in pigeons.

CONCLUSIONS

Normal blood counts on a large number of pigeons are reported, and the great variability previously reported confirmed.

REFERENCES

- ¹De Eds and Mitchell. *Jour. Pharm. Exp. Therap.*, 1926, *xxviii*, 433.
- ²Klieneberger and Carl. *Die Blutmorphologie der Laboratoriums Tiere*, 1912, Leipzig.
- ³Arloing and Dufourt. *Soc. sc. vet., Jour. de méd. vet. et de zootech.*, Lyon, May, 1922.
- ⁴Hastings. *Bull. Johns Hopkins Hosp.*, 1904, *xx*, 122.
- Rees and Ecker. *Jour. Am. Med. Assn.*, 1923, *lxx*, 621.

A STUDY OF THE PIGMENT IN ADDISON'S DISEASE*

By CARL L. SPONK, M.D., AND ROBERT A. MOORE, A.B. COLUMBUS, OHIO

MELANIN occurs normally as the coloring matter of the hair, of the choroid of the eye, of the skin and in the pigment matter of many lower animals.

Its function is evidently that of protection from light rays. Melanin seems to be produced through the metabolic activity of specialized cells and old theories concerning its origin from hemoglobin have been abandoned.

Because of some unusual activity, melanin accumulation may occur somewhere within the body in excess. While the entire skin of a negro is said to contain only about one gram of melanin, excessive quantities may be deposited in the lymph nodes, skin, etc.

Addison's disease is usually associated with a deposition of a brownish pigment in the skin and occasionally in other organs. Von Furth and his coworkers have shown a definite relationship of tyrosine and melanin and other investigators have, at least partially, shown a relationship of tyrosine

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.
From the Department of Pathology, the Ohio State University, Columbus, Ohio.

and adrenalin. On this basis it has been assumed that the pigment of use in Addison's disease is a melanin and is due to some dysfunction in the metabolism of the aromatic radicles. As yet no definite chemical evidence has been brought forward to prove the melanotic character of this pigment.

During the spring of 1925 it was our good fortune to perform a necropsy (on the pathologic service of the Ohio State University) on a typical case of Addison's disease with pigmentation of the skin and active tubercular caseation of the right adrenal and old tubercular fibrosis of the left adrenal. The point of greatest interest was that the entire lymphatic system of the abdomen and chest was heavily pigmented black. The case report and histologic findings will be reported elsewhere, suffice it to say at this time that it was early realized that the pigment was not an anthracosis. Microchemical reactions showed that the pigment was non free and was bleached by sunlight and hydrogen peroxide. In accord with this idea a chemical investigation of the pigment was thought to be pertinent.

Isolation of the Pigment—The method of Gortner² using 0.2 per cent NaOH was followed very closely. In brief this method is as follows. The lymph glands were finely ground in a mortar and placed in a flask with an excess of 0.2 per cent NaOH. The whole was boiled under a reflux condenser for three hours. The supernatant fluid was poured off and the process of extraction repeated. The two extracts were combined, filtered through a Buchner filter and concentrated. HCl added until a coarse flocculent precipitate appeared. The whole was centrifuged and the supernatant poured off. The dark mass remaining was dissolved in N/20 HCl and again centrifuged and the melanin solution poured off. Concentrated HCl was added until a flocculent precipitate appeared. (Gortner recommends adding up to 1 per cent but we find that this is not sufficient acid to cause the precipitation of the melanin. Titration results on the fluid show the concentration to be about 2.5 per cent.) The precipitate is centrifuged and the acid poured off. The precipitate is dissolved in an excess of 50 per cent acetic acid and centrifuged. The dark supernatant solution is placed in a celloidin dialyzing sac and dialyzed until the melanin is precipitated and the dialyzate is free of chlorides. Centrifuge the suspension and collect the precipitate in a porcelain dish. Dry on a water bath. The dry black powder is extracted in a Soxhlet with carbon bisulphide, alcohol and finally ether. This dry powder was used in the analytical results reported below.

Amount of Pigment—Unfortunately the lymph glands were not weighed at the start and only a very general idea of the amount of pigment can be secured when it is stated that the entire lymphatic apparatus of the thorax and abdomen yielded about 0.7 gm. of pigment.

Ash Content—The pigment was placed in a platinum crucible and heated until all organic matter was oxidized.

0.126 gm. of pigment yielded 0.0011 gm. of ash, or 0.93 %

Sulphur—Liebig's alkali method was used as outlined by Sherman.³ An alcohol lamp and nickel apparatus was used for the fusion. The barium sulphate was washed, ignited and weighed as outlined by Foulk.⁴

0.3054 gm of pigment yielded 0.0683 gm of barium sulphate. Calculated on an ash free basis this is 3.10 per cent S (0.0032 gm of barium sulphate was secured in a blank).

Nitrogen—This constituent was determined by the general Kjeldahl method outlined by Foulk.⁴

0.0174 gm of pigment gave off the ammonia equivalent of 5.48 cc of N/50 HCl.

Calculated on an ash free basis this is 11.24 per cent considering that 1 cc of 0.02 N ammonia equal 0.000341 gm ammonia.

Carbon and Hydrogen—These two elements were determined in a combustion train according to the methods outlined by Gatterman⁷ for substances containing nitrogen and sulphur. The combustion was carried out with lead chromate and a reduced copper spiral.

0.2150 gm pigment yielded 0.1064 gm water and 0.4287 gm carbon dioxide. Calculated on an ash free basis this is

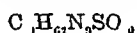
C	54.8 per cent
H	5.56 per cent

SUMMARY

From these results we can formulate

C	54.8 per cent
H	5.56 per cent
S	3.1 per cent
N	11.24 per cent
O	25.4 per cent (by difference)

Calculated on this basis the simplest possible formula is



DISCUSSION

As these results appear to us, the exact amounts of each substance are only of value to a slight extent for as has been pointed out by Gortner² it is impossible to prepare two samples of melanin from the same source and secure the same figures.

The significance of these results lies mostly in the sulphur determinations. Granting that the Liebig alkali method gives consistently high results even on known chemical compounds, the value of 3.10 per cent after a blank determination has been subtracted points most clearly to the fact that this pigment is not related to any known derivative of hemoglobin formed either by changes in the animal body or by chemical processes outside the body, since none of these derivatives have ever shown a higher percentage than 0.5 per cent of S.

In the isolation of the pigment it was noted that the two types of melanin mentioned by Gortner² were present, that is one soluble in weak acid and one insoluble in weak acid. The amount of the latter was so small that any analytic work was impossible.

The comparison of the results of the chemical analysis of this pigment with that of melanins derived from other sources by different authors is not of any value. As has been pointed out by Gortner² the actual chemical percentages of the elements vary considerably with the method of extraction. But

since we have followed the method used by Gortner in detail, it follows that a comparison with the results of his analysis using 0.2 per cent NaOH to extract the pigment from black wool is justifiable

	C	H	N	S
Gortner	52.60	7.28	13.52	1.33
This paper	44.8	5.56	11.24	3.10

Although the differences are well beyond the limits of experimental error and beyond the errors secured by us in the analysis of known chemical compounds with the same apparatus and chemicals, we believe that, considering the possibility of slight variations in the technique of purifying the pigment and in the possibility of several different melanins existing, the figures are comparable, with the exception of the sulphur. Here some of the differences may be accounted for on the basis of the Liebig method used by us, but the remainder must remain unaccounted for until further observations can be made in other cases of Addison's disease.

SUMMARY

1 The ultimate chemical analysis of a pigment derived from the lymph glands in a case of Addison's disease is reported.

2 The evidence seems to point that this pigment is a melanin and the absence of any other explainable cause of the melanosis than the Addison's disease would lead one to believe that there was more than a casual relationship between the two.

3 We believe that the pigment laid down in the tissues is a melanin and that it bears a direct relationship to the condition responsible for the pathologic complex of Addison's disease.

REFERENCES

- ¹Abel and Davis *Jour Exp Med*, 1896 : 361
- ²Gortner *Biochem Bull* 1911 : 307
- ³Sherman *Organic Analysis*, Macmillan Co. New York
- ⁴Foulk *Notes on Quantitative Chemical Analysis*
- ⁵Gatterman *Practical Methods of Organic Chemistry*, Macmillan Co. New York
- ⁶Wells *Chemical Pathology*, ed 5 W B Saunders Company, Philadelphia, Pa.
- ⁷McCallum *Textbook of Pathology*, ed 3

DISCUSSION

Dr Wm G. Exton—It really represents a tremendous amount of work. I am not quite clear, Dr Spohr, about the final figures isn't that a little out of the way?

Dr Carl Spohr—Our figures were higher than those given by Gortner.

Dr Wm G. Exton (continuing)—I wonder if that is close enough. We got consistent results showing that the method by Gortner was a good one. What I wanted to do was to call attention to the vast amount of work in this

EXPERIMENTAL BACILLUS PYOCYANEUS KERATITIS²

BY EDNA JACKSON, M A, AND F W HARTMAN, M D, DETROIT, MICH

THE pathogenicity of *B. pyocyaneus* for laboratory animals is well recognized but it is generally regarded as of low virulence in man. There are recorded, however, a few isolated eye infections of most serious character. As in the reports of Mauersberg, Kritzky and Lamb and Calhoun, several of the observers have reproduced the lesion in the eyes of rabbits.

The virulent strain of *B. pyocyaneus* used in this study was isolated from seven of a group of eighteen industrial cases. The detailed report of these cases and the review of those appearing in the literature will appear elsewhere.

Of the eleven eyes from which *pyocyaneus* was not isolated, *staphylococcus aureus* was isolated in two, *staphylococcus albus* in two, two cultures were negative and five were not cultured for various reasons. After two successive cultures showed *pyocyaneus* the infected swabs were used to injure the cornea of rabbits' eyes in an attempt to reproduce the disease. This attempt failed and further experiments included six groups, all under cocaine anesthesia, as follows:

1 *Injection of the culture between the layers of the cornea*—As the rubbing of the cornea with an infected swab gave no results, and as the clinical cases gave history of injury to the eye before infection, infection was attempted by an inoculation which would injure the cornea and place some of the organisms between the corneal layers. The growth from a twenty-four hour agar slant culture of *B. pyocyaneus* was washed off with 5 c.c. of sterile saline. By means of a fine hypodermic needle, inoculations of this suspension were made into the cornea of both eyes of two rabbits and of one guinea pig. A lesion about 2 mm. in diameter was thus produced. At the end of twenty-four hours there had developed from each inoculation a marked conjunctivitis, and at the site of each inoculation an ulcer had developed which already showed marked digestion. Pus was present in quantities, making the opening of the eyelids difficult. In forty-eight hours the ulcers had spread over a much larger area, the affected part being opaque, showing digestion and sloughing of the tissue. At the end of four days the ulcers involved the entire cornea in the rabbits, and in the guinea pig so much sloughing had occurred that the anterior chamber of the eye had evacuated itself. Cultures taken from the ulcers at the end of three days showed *B. pyocyaneus* in pure culture.

Control sterile needle punctures into the cornea caused a slight conjunctivitis which quickly subsided, and at the end of twenty-four hours the point of puncture was scarcely visible.

²Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.
From the Laboratories of the Henry Ford Hospital, Detroit, Mich.

TABLE I

EX. I INJECTION OF CULTURE OF *B. PYOCYANEUS* BETWEEN LAYERS OF CORNEA

	24 HOURS	48 HOURS	4 DAYS
Rabbit No 1	Large spreading ulcers Conjunctivitis Pus	Large deep spreading ulcers Conjunctivitis Profuse pus formation Sloughing of cornea.	Deep undermining ulcers covering entire visible portion of eye Conjunctivitis Pus
Rabbit No 2	Large spreading ulcers Conjunctivitis marked Profuse pus formation	Large ulcers spreading Conjunctivitis Profuse pus formation Affected portion opaque	Deep ulcers covering en- tire visible portion of eye Conjunctivitis Pus Whole cornea opaque
Guinea Pig No 3	Ulcers rapidly spreading Marked conjunctivitis Profuse pus formation	Ulcers spreading Conjunctivitis Profuse pus formation Digestion marked Sloughing of cornea	Ulcers covering entire visible portion of eye Marked digestion and sloughing Large amount of pus and exudate
Rabbit No 3 a Control sterile needle puncture	Very slight conjunctiv- itis.	Negative	Negative

2 *Instillation of pyocyaneus cultures into the conjunctival sac*—Another attempt was made to produce infection by instilling a saline suspension of a twenty four hour culture of a virulent strain into the conjunctival sac of the eyes of two rabbits. At no time could any effect from these instillations be observed.

3 *Scratching the cornea and instilling the culture into the conjunctival sac*—In this experiment six rabbits were used. In three corneal layers of eyes were scratched with a needle and in three the corneal layers were scratched by means of fine iron filings. One of each set was kept as a control. Cultures of *B. pyocyaneus* were instilled into the conjunctival sac of the others. Where iron filings were used the results were negative. Where the cornea was scratched with a needle a slight conjunctivitis developed which disappeared in two or three days. No ulcers formed and the injuries were scarcely visible in twenty four hours.

4 *Injection of broth filtrate into the corneal layers*—Questioning the role that the proteolytic ferments present in pyocyaneus filtrates might play in the production of these digesting ulcers we injected sterile broth filtrates from six day old cultures into the cornea of both eyes of two rabbits. The inoculations were made by means of a hypodermic needle as in the first experiment. From these inoculations there resulted a marked conjunctivitis also slight pus formation. No ulcers formed and there was no evidence of digestion. The conjunctivitis subsided and in four days the eyes appeared normal except for the minute lesions at the site of inoculation.

5 *Inoculations of Staphylococcus aureus and Staphylococcus aureus plus sterile broth filtrates of B. pyocyaneus*—The staphylococcus culture used in the experiment was one which had been isolated from one of the severe clinical cases. Saline suspensions were made from a twenty four hour agar slant culture and needle inoculations were made into the corneal layers of both

eyes of two rabbits In twenty-four hours there was a marked conjunctivitis, a moderate amount of exudate present and small staphylomas at the points of inoculation These lesions showed no digestion and there was no extension of the lesions In four days the eyes of one of the rabbits were apparently normal, while one of the eyes of the other rabbit showed a small staphyloma There was no evidence of digestion or of a tendency to spread



Fig 1—Staphyloma at the site of inoculation with conjunctivitis

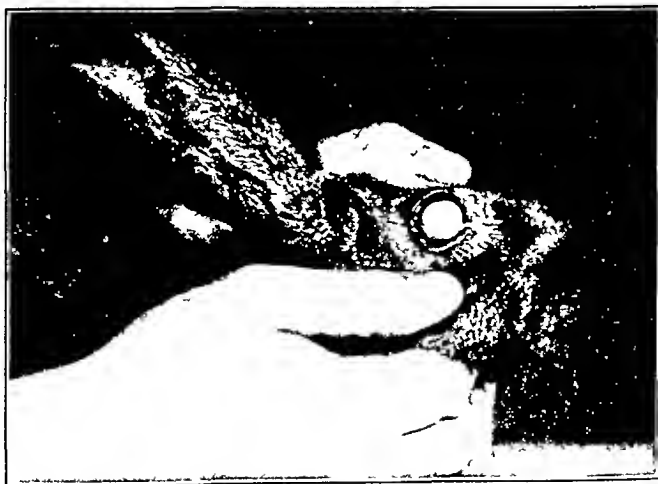


Fig 2—Shows complete undermining and opacity of cornea

It seemed of interest to determine whether the staphylococcus could, in the presence of the pyocyaneus filtrate, produce lesions similar to those produced by the inoculation of *B pyocyaneus* The eyes of two rabbits were injected as above, using a mixture of equal parts of the saline suspension, of staphylococcus and of sterile filtrate from pyocyaneus broth cultures

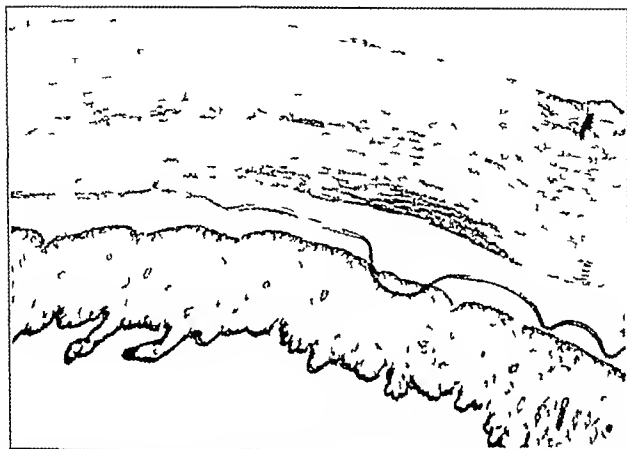


Fig 3—Microphotograph with $\frac{1}{5}$ objective showing moderate diffuse infiltration of the corneal layers with separation of the same



Fig 4—Microphotograph with $\frac{1}{6}$ objective shows partial separation of Bowman's capsule and intense infiltration of the layers of the cornea. The adjacent eyelid is also infiltrated with round wandering cells and polymorphonuclear leucocytes

In these cases the resulting conjunctivitis was more marked than when staphylococci were injected alone, there was also more exudate present and evidence of slight digestion. The lesions, however, did not spread and gradually the conjunctivitis subsided so that in a few days the only evidences of the infection were small lesions at the points of inoculation.

6 *Injection of old laboratory strains of pyocyaneus*—Two old laboratory strains of pyocyaneus were obtained through the courtesy of Parke Davis & Company. Saline suspensions of twenty-four-hour old cultures were injected into the corneal layers of two rabbits. The lesions following injection of one strain showed considerable digestion and marked conjunctivitis, but no typical ulcers developed and there was no extension of the lesions. The conjunctivitis was marked for several days, then it gradually subsided. The lesions



Fig 5.—Shows microphotograph with $\frac{3}{4}$ objective. Intense infiltration of the cornea and exudate in the anterior chamber of the eye.

showed no ulceration or extension. The reaction from inoculation of the second strain was less marked. There was a slight conjunctivitis and small localized lesions which showed no digestion and no spreading. These laboratory strains possess only a low virulence for guinea pigs, a half c.c. of saline suspension injected intraperitoneally into 250 gm guinea pigs did not kill. The animals were somewhat affected for two days but all recovered. The injection of $\frac{1}{20}$ c.c. of our freshly isolated strains always killed within two days. Attempts to raise the virulence of the laboratory strains by means of passage from one guinea pig to another were not successful.

Whether or not there is any relation between virulence and pigment fermentation, it is interesting to note that in the old laboratory strains there was no chloroform-soluble bluish-green pigment present. The agar slant cul-

TABLE II

EX. VII IMMUNIZED RABBITS INJECTED WITH CULTURES OF *B. PYOCYANEUS*

	24 HOURS	48 HOURS	4 DAYS	PROTEC TION
Rabbit No 19 (Immunized with <i>B. pyocyaneus</i> cultures.)	Marked conjunctivitis Small lesions Pus present No digestion No extension	Marked conjunctivitis Small localized lesions Small amount of pus	Little conjunctivitis Very slight lesion at points of inocula- tion No pus	Marked
Rabbit No 20 (Immunized with <i>B. pyocyaneus</i> cultures.)	Marked conjunctivitis Small amount of pus No digestion	Conjunctivitis subside ing Lesions small not ex- tending	Little conjunctivitis Small localized le- sions at point of inoculation	Marked
Rabbit No 21 (Immunized with <i>B. pyocyaneus</i> filtrate.)	Marked conjunctivitis Small lesions Pus present No digestion ob- served	Marked conjunctivitis Lesions somewhat ex- tended Large amount of pus	Conjunctivitis less Lesions show no fur- ther extension Small amount of pus No sloughing	Moderate
Rabbit No 22 (Immunized with <i>B. pyocyaneus</i> filtrate.)	Marked conjunctivitis Lesions small Large amount of pus	Marked conjunctivitis Lesions somewhat ex- tended Large amount of pus No deep ulcerations	Conjunctivitis subside ing Lesions localized no further extension— larger than in Rab- bits 19 and 20	Moderate
Rabbit No 23 Control	Marked conjunctivitis Definitely spreading ulcers Large amount of pus	Marked conjunctivitis Marked digestion Large amount of pus Ulcers extending	Marked conjunctivitis Visible portion of eye opaque Large amount of pus Sloughing	

TABLE III

EX. VIII RABBITS INJECTED WITH CULTURES OF *B. PYOCYANEUS* AND RECEIVING SERUM FROM IMMUNIZED RABBIT

	ANTI CULTURE SERUM	24 HOURS	48 HOURS	4 DAYS
Rabbit No 24	2 cc iv 18 cc at end of 24 hours	Marked conjunctivitis Much pus Lesions slightly enlarged	Marked conjunctivitis Small amount of pus No extension	Slight conjunctivitis No pus Small lesions at point of inoculation
Rabbit No 25	2 cc iv 1 cc 24 hours	Marked conjunctivitis Large amount of pus	Marked conjunctivitis Less Some extension of le- sion in left eye	Slight conjunctivitis Pus No further extension of lesion No erosion
Rabbit No 26	Control	Marked conjunctivitis Large amount of pus Some digestion	Marked conjunctivitis Large amount of pus Ulcers spreading Digestion marked	Conjunctivitis. Large amount of pus Visible portion of eye opaque Sloughing

tures appeared somewhat fluorescent but they lacked the typical bluish green pigment of the freshly isolated cultures. Agar slants of these cultures are distinctly bluish green and the pigment is easily extracted by chloroform.

7 Since clinically the only effective treatment tried was actual cautery it seemed worth while to investigate the possibility of active and passive immunity—For this purpose two groups of rabbits were taken. One group was injected with increasing doses of a killed culture of *B. pyocyaneus* the other with sterile filtrates of six day broth cultures of *B. pyocyaneus*. The killed

cultures used were obtained by washing the growth from a twenty-four hour agar culture, then washing this once with sterile saline. The sediment was taken up in sterile saline and heated at 60° for forty-five minutes. Because of the difficulty often encountered in giving injections of pyocyaneus, small doses were given, beginning with doses of 0.2 c.c. of the culture and filtrate, slowly increasing the amount until 0.75 c.c. was given in the eighth injection. After the eighth injection samples of blood were taken from one of each group to test the titer of the serum. The serum of the rabbit which had received the culture injections completely agglutinated the pyocyaneus organisms in a dilution of 1-640, incompletely in a dilution of 1-1280 (two hours in the water-bath and overnight at room temperature). The serum from the rabbit which had received the injections of filtrate was tested against a sterile



Fig. 6—Microphotograph with $\frac{1}{6}$ objective same area showing character of infiltration of the cornea

broth pyocyaneus filtrate for the presence of precipitins and against saline suspensions of pyocyaneus for the presence of agglutinins. No precipitin reaction was obtained but the serum agglutinated the pyocyaneus culture in a dilution of 1-320. These rabbits were bled and their serum used in an experiment described later.

Two rabbits which had received the injection of killed cultures and two which had received the filtrate injections were inoculated with a twenty-four hour culture of pyocyaneus, a control rabbit being injected at the same time. These inoculations were made by hypodermic needle into layers of cornea as before.

The control rabbit developed the typical spreading lesions which have been described above. The purulent conjunctivitis persisted at end of two weeks. The ulcers extended gradually, covering the cornea. The rabbits

immunized by killed cultures developed, after the inoculation of the eyes, a marked conjunctivitis, lesions formed at site of inoculation but these showed little tendency to spread. There was no ulceration. At the end of four days the conjunctivitis had about subsided, there remained only very small lesions at the site of inoculation. The rabbits which had been inoculated with the sterile filtrate from broth cultures of pyocyanus developed more destructive lesions than those which had been immunized with cultures.

From this small number of animals it would seem that a better immunity was obtained by injection of cultures than by injection of filtrates.

The two rabbits whose serum had been tested for agglutinating titer were bled to death, the serum being used to passively immunize other rabbits.

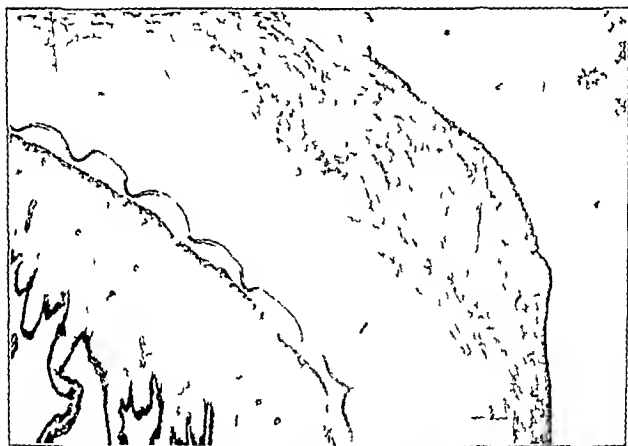


Fig. 1.—Shows microphotograph with $\frac{1}{4}$ objective with area of dense scarring in the cornea.

Three rabbits were injected with a twenty-four hour culture of *B. pyocyanus* by needle inoculation into the cornea. One of the rabbits inoculated was given 25 c.c. of serum from the rabbit which had been immunized by injection of killed cultures. In twenty-four hours there was little difference to be noted in the reactions in the two rabbits. (A second intravenous injection of 20 c.c. of serum was given.) In forty-eight hours the eye of the control rabbit showed marked conjunctivitis, profuse exudation, the lesions were spreading rapidly. In the rabbit which had received the immune serum the lesions were small and they showed no extension. The conjunctivitis which had been so marked was receding and there was little exudate present. At the end of four days the conjunctiva was about normal, there was no pus and the lesions at the site of inoculation were very small and definitely localized. The lesions of the control rabbit had extended, showed more disintegration and there was still

a large amount of pus present. The third rabbit received 20 c c of immune serum from the rabbit which had received the injection of sterile broth filtrate and 18 c c again at the end of twenty-four hours. In this rabbit little evidence of protection was noted. The lesions which developed were of the same severity as those of the control, the spreading of lesions and digestion of tissue did not seem to be affected by the serum.

SUMMARY

1 Keratitis was experimentally produced by *B. pyocyaneus*, the inoculations being made into the corneal layers.

2 Attempts to produce keratitis were not successful.

(1) When instillations of *B. pyocyaneus* were made into the conjunctival sac.

(2) When *pyocyaneus* filtrates were injected.

(3) When cultures of staphylococci were injected into corneal layers.

(4) When cultures of old laboratory strains were injected into corneal layers.

3 Rabbits were effectively immunized by injection of killed cultures of *B. pyocyaneus* and by injection of *pyocyaneus* filtrates.

4 Rabbits receiving immune serum were not effectively protected except in one case. Favorable results in this case suggest that an effective immune serum could be produced.

REFERENCES

- Lamb, H. D., and Calhoun, J. G. *Am Jour Ophth*, 1916, *xxiii*, 257.
Krintzky, A. D. *Vertrnik Oftalmologii*, 1911, *xxviii*, 945.
Jacobi, Paul. Heidelberg, 1912.
McNab, Angus. *Ophthal Rev*, 1904, *xxiii*, 65.

INTESTINAL AMEBIASIS FROM THE PATHOLOGIST'S STANDPOINT AS RELATED TO THE CLINICAL PICTURE*



BY J. M. FEDER, M.D., GRAND JUNCTION, COLO.

INTESTINAL amebiasis was long considered a disease almost wholly tropical, very few cases occurring within the temperate zone were recorded, however, within the past decade the number of cases manifesting themselves is steadily rising. That this disease is by no means a rarity in the temperate zone can be borne out by the figures of Kotold and others which showed that 3 per cent of all soldiers serving in the United States were infected with *E. histolytica* and that 108 per cent of those returning from France were similarly infected. Then, recognizing that intestinal amebiasis is a cosmopolitan disease it is felt that we must be more and more on the alert to detect it, both in the patient suffering from the disease and in the carrier. In the preparation of this paper we have constantly kept the clinical pathologist in mind as the pivot upon whom the control of this condition must turn and to whom the clinician will come with his problems of differential diagnosis and later for treatment control. We have endeavored to keep constantly in view the fact that this is not a symposium on protozoology or pathology but rather an attempt to crystallize into as short a space as possible, this as a practical subject in a manner acceptable to practical men.

We have purposely omitted all cumbersome data and have boiled the text down to proved facts. Charts and specimens have been used where it is considered that they would replace a lengthy description. Many facts and figures herein included have been drawn from the experiences of others and as the sources have been so numerous it is not possible to give due credit in each case. No one was in a better position to study this condition than the pioneer physicians in the Panama Canal Zone and their work will endure for all time as a most valuable contribution to protozoology. Those physicians found amebiasis rampant during the early days and soon found that its immediate reduction or eradication was imperative. I recently heard a local authority down there state that at that time intestinal amebiasis was one of the most widely spread protozoal diseases and that malaria was not excluded.

We will now endeavor to demonstrate that with the coming of sanitation the number of cases on the Canal Zone dropped to almost zero. It is noted that there is a great decrease in the number of cases coming to autopsy at the Ancon Board of Health Laboratory from 1905 to 1923. It will be noted that the cases have fallen from 50 cases of amebiasis to the 1000 autopsies in 1905 to 4 cases to the 1000 in 1923. Undoubtedly the coming of better

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 1, 16 and 17, 1926.

hygienic conditions was responsible for this drop on the Canal Zone. At the Santo Tomas Hospital in the Republic of Panama where our patients are drawn from the interior, the fall in late incidence has not been so spectacular. Reliable statistics are not available for the same period in this institution but at present about 2 per cent of all cases coming to autopsy show gross lesions of amebiasis, many more microscopically.

Now that we have demonstrated that the number of cases fell in ratio to improved sanitary measures, let us consider the mode of transmission in order to more clearly appreciate the hygienic procedures required.

Man can become infected with *E. histolytica* in only one way, and that is to swallow the cysts of the organism, and the consensus of opinion among us is that those cysts are ingested by the accidental contamination of food or drink by minute particles of feces containing them and that while the particle of feces may be dry on the surface, its moist center protects living cysts. It is believed that the carrier problem is the biggest factor in the transmission of the disease and that contaminated water supplies play a minor rôle if any. It has been the experience in Panama that cases following flooding of wells and other known contamination of water supplies are rare and that the dysenteries following such contamination are usually bacillary in origin. In a country where amebiasis is endemic, one would naturally expect to find some epidemics following these accidents if the disease were easily transmitted by water supplies. We do at times, however, find the two dysenteries existing in the same individual following these exposures, but as an attack of bacillary dysentery frequently brings about an acute exacerbation in a case of chronic amebiasis, little import is attached to them from the standpoint of epidemiology.

The opportunity to prove the carrier theory has been given many times, for instance in the practice of a prominent local physician, several children in a wealthy family were suddenly seized with severe amebic dysentery. There was no history of suspicious food or water and the children had not been out of the sanitized area. Upon examining the servants for a possible carrier, one maid was found who was passing cysts of *E. histolytica* in large numbers. Flies are also looked upon as potential carriers and no doubt are responsible for the transmission of many cases. Another prolific source of infection is the Chinese gardener with his luxurious green vegetables, made so by the daily dipper of night soil. We must regard all green vegetables as infected and as a rule one does not eat local vegetables uncooked. I know of no means whereby one can rid them of the potential danger of being harboreis of amebic cysts and at the same time preserve the palatability of the article.

In striking at the root of this problem then, we must hunt down the carrier just as we do in typhoid and kindred infections, and we must consider the examination of all food handlers in endemic centers and it is felt that this point cannot be overemphasized. You will find the lower class native to be filthy and careless, this individual may be symptomless himself but a potential source of danger to those about him. Of course, everyone who

ingests cysts does not become infected as was proved by the classical experiments of Walker and Sellards at Bilibid Prison in Manila. Were such the case, everyone residing in the endemic centers would probably be infected as one has but to see the toilet facilities in the outlying districts to realize that obtaining particles of cyst containing material along with one's food is far from impossible. A recent survey of the waiters at Santa Marta Columbian Hospital of the United Fruit Company, showed 60 per cent infected. At this time it is deemed desirable to emphasize a few important points readily recognized by tropical workers but as a class given scant attention by writers on general medical subjects.

First Any dysentery may be of amebic origin but we recognize about thirteen other etiologic factors and the task of their differentiation falls to the lot of the laboratory worker in most instances.

Second That amebiasis may exist without dysentery. That constipation may be present instead of diarrhea and that the infestation may only make itself known by vague gastrointestinal symptoms. The point that I wish to especially emphasize here is that dysentery is *not* the commonest manifestation of intestinal amebiasis.

In the acute cases with the active ameba the diagnosis will be easy and the ordinary examination will readily detect the organism but in the chronic and carrier state other means must be resorted to. The stained specimen of stool offers the only solution to this problem. The technique can be obtained from any textbook on protozoology but the technical work is not easy and the differentiation of the cysts is fraught with difficulty. We are gradually awakening to the fact that we have been heretofore missing from 10 to 40 per cent of our cases for the very reason that we were not making these wet fixed and stained preparations. Time does not permit me to go into the life cycle of the organism or methods of differentiation.

Another point of considerable importance to be emphasized is the fact that intestinal amebiasis is a protean disease manifesting itself in many ways. Down in the tropics it is well said that given a case of a vague condition of any kind where one has reason to suspect exposure to amebic infection that condition must be included or excluded before a final opinion is given. And as has been stated before all residents of endemic centers are exposed to infections in fluences as were also many of our soldiers who served in France. It is well within the bounds of possibility that many cases are being passed without being diagnosed in parts of the country where only a rare case is seen. They are frequently missed down there where everyone should be suspected. One may often find a small liver abscess responsible for fever of unknown etiology, the liver abscess being too small to detect by ordinary methods of physical examination. Vague cerebral symptoms may be due to amebic abscess of the brain and an amebic typhilitis can accurately mimic an acute appendicitis. A lung abscess secondary to a liver abscess can closely simulate lobar pneumonia and a failure to make a prompt diagnosis will cost a life.

In examining material from liver abscesses we have found that only rarely are we successful in locating the ameba in the first fluid aspirated from the abscess or from the first gush of pus after opening. The amebae are

not free in the pus but imbedded in the walls of the abscess and can be found in the discharge several days after opening. We have found it indeed poor policy to scrape the abscess wall to obtain material for diagnostic or other purposes as for some at present unaccounted for reason, the slightest manipulation of the abscess cavity has been followed by grave results and usually death. We do not swab out the cavities any more but are satisfied with merely opening and draining.

I promised you in the beginning that this article would be as brief as would be consistent with conveying to you a few practical chapters from our experience. Therefore, I have purposely omitted any reference to microscopic pathology or morbid anatomy. These can be found in a text on tropical medicine.

From the extensive damage done in some of these intestinal cases, one must at once be impressed that those cases that go on to recovery must have suffered so much damage that it would not be possible to bring about a cure in a short time. There may be a symptomatic cure but we must follow up these cases and make repeated examinations to determine that they are free from organisms. Before closing, permit me to stress another point, that is, a suspected case may have to be examined several times before the organisms are found as our approximate statistics show that in only 64 per cent of the positive cases was the first examination successful. According to the best authorities, we will miss from 10 to 40 per cent of our latent cases if we do not resort to the wet-fixed, stained preparations.

CONCLUSIONS

1 This is not meant as an exhaustive treatise upon the subject presented, but merely an effort to summarize some of the work that we are now doing and to demonstrate what has been done.

2 That improved sanitation has been responsible for a sharp decline in the number of cases of amebiasis.

3 That intestinal amebiasis is no longer to be considered as a purely tropical disease, but rather one of a cosmopolitan character.

4 That dysentery is not at all a necessary accompaniment of intestinal amebiasis and it is in the vague case without dysentery that the most skill in examination of the stools is required.

5 That there is a carrier problem and that it is a vital factor in the spread of the disease.

6 That amebiasis is an extremely protean disease and every case of vague gastrointestinal upset must be looked upon with suspicion.

Finally, I wish at this time to express my appreciation for the aid given me by all of the physicians on the Canal Zone for their assistance in compiling this data. To the pioneers I am extremely grateful for without their work this record of improvement could never have been.

DISCUSSION

Dr Isaac J. Jones—I don't know that I have anything to offer that would be of any value but I am likewise interested in this subject. I lived in the tropics for fifteen years and spent ten months on the Isthmus itself. There was one statement that rather surprised

me, that is that amebiasis is not ordinarily a water borne disease. I had one experience personally. In investigating an epidemic of amebic dysentery the people obtained their water supply from a spring at the edge of the town. There was no possibility of an infection from the spring from watershed. In observing the place I found that the women of the town came there to get water they were all barefoot. They would walk up to the edge of the spring and draw the bucket through the water, and as the bucket was drawn out the water would wash out over the feet and flow back into the spring.

Dr Kenneth M. Lynch—Undoubtedly the prevalence of amebiasis varies in different regions, probably with the sanitary conditions. In South Carolina I found more than I do in Texas. The only active cases I have seen in Texas have been imported from Mexico or Central America. The local cases have been inactive or carriers. There is one proposition I want to recall in connection with the spread of the disease the possibility of the rat as a carrier. I was able to show that such could be the case and that it actually happened at least in one focus in 1915. This has been amply confirmed in an experimental way and should be kept in mind.

The second question which I want to discuss is that of the possible and probable common mistake in diagnosis. I have not been so concerned about the reported incidence of histolytica being too low but rather too high. It is around one per cent in this region in over a thousand cases I have studied. It is easy enough to mistake coli in free active stage for histolytica. I have seen any number of examples of this mistake and have come to pay little or no attention to an identification of an intestinal ameba except in the encysted stage. I will not make such a diagnosis when the ameba is in the free active stage but I call for a formed stool and wait for the cysts to appear. A fresh stool is not necessary in the identification of amebas a fresh warm purged stool leads to mistakes instead. You must find the cyst or your diagnosis must be questioned except of course in the case of clinical dysentery, when a working diagnosis can be made very properly.

Dr A. H. Sanford—The incidence of amebiasis as reported at the Mayo Clinic may be the subject of some comment. I appreciate that there is considerable difficulty in differentiating between *E. coli* and *E. histolytica*.

The incidence of histolytica in our reports may be too high. It must be remembered however that stool examinations are not made routinely. Only those patients with intestinal symptoms are sent to the laboratory for this examination. This would account to a great extent for the high proportion of findings of amebas of the pathogenic type. Since 1919 Dr Magath a trained parasitologist has been studying this problem and it is not settled yet. There has been a great deal of controversy concerning amebiasis and there will be still more before the truth is known. Regarding Dr Feder's contention that diagnoses should be made only with stained fixed wet specimens I would admit that he may be right. However amebas are usually found by examining the fresh stool directly with a cover glass preparation and a tentative diagnosis made. The one thing that I do want to emphasize is that the men who are here from the north should get busy and examine stools, we have amebiasis in the northern states.

Dr H. S. Thomas—Within the last three years we have examined six thousand specimens of stool for protozoa. Eleven hundred of these were inhabitants of New York State and in these cysts of *E. histolytica* were found in 1.6 per cent. None had symptoms of amebic disease. On account of the low figure and the complete absence of symptoms in the patients harboring the cysts we have refused to become excited about amebic disease in the northern states in spite of the fact that an occasional case undoubtedly occurs.

Dr T. C. Terrell—I think a great deal has to do with the specimen being properly collected. Where possible we have the patient come to the laboratory and take a good big dose of salts and remain at the laboratory until satisfactory specimens are obtained. We collect at least the first and second specimens and more if necessary, thereby giving us the opportunity of studying both the formed and liquid stools as the first stool is usually at least partially formed. Of course in the cases where the salts is not necessary we do not put them through that routine.

A PHOTOGRAPHIC METHOD OF COUNTING BLOOD CELLS*

BY ARTHUR H. SANFORD, M.D., ROCHESTER, MINN.

THE computation of the cellular elements of the blood has been a clinical laboratory procedure for fifty years. From time to time refinements in methods of technique have been added, but we think of Gowers,¹ Hayem,² Lyon and Thoma³ and others as having established the value of hemocytometry, and of having pointed out some of the inherent difficulties in the procedure. The normal count of erythrocytes was early established at 5,000,000 for the normal adult male.

The usual textbook method for blood counts, also advocated by the investigators of fifty years ago, involves the making of several counts and determining the average. While this procedure is ideal, in practice it is often neglected when the volume of work is such that time is an essential factor, or when the results are normal, or what was expected. There are numerous chances for slight error in as simple a procedure as an erythrocyte count. Various attempts at evaluating the probable error have usually placed it at about 2 or 3 per cent, and always less than 5 per cent. These errors may be due to faulty pipettes, faulty counting-chambers, faulty technique in taking the blood, in drawing it into the pipette, or in making the dilution or in filling the chamber. Recognition of all of these sources of error was responsible for many of the improvements in apparatus.

Another source of error, however, may be in the actual counting of cells as seen in the microscopic field. While the average person should easily learn the technique of counting blood, the fact still remains that there is a considerable personal error in counting up to 500 cells in eighty small squares when the dilution of blood is 1:200. In order to reduce this error to a minimum and to have a permanent record of a count, the photographic method was devised. This method has not been recommended for routine work, although there is no reason why it could not be used as a routine if it should seem advisable.

The apparatus (Fig. 1) was readily assembled. It was necessary to keep the counting-chamber horizontal, and desirable to have a strong light below the stage. The Spencer micoprojector, Number 9100, was found to be most suitable for this purpose. This was fastened securely to the framework constructed in the manner illustrated, and an ordinary view camera with a draw of fifteen inches suspended above the microscope of the projector. The total distance from the stage to the back of the camera is twenty-four inches. The eyepiece 6x used with an 8 mm. objective and this camera draw gave a good field of more than 100 small squares of the counting chamber. The magnifica-

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 15, 16 and 17, 1926.
From the Section on Clinical Pathology, Mayo Clinic, Rochester, Minnesota.

tion is about 200. The negatives are made on process films and prints made, or the prints can be made directly on photostat paper. Two seconds was found to be the optimum exposure for either type of picture.

The counting apparatus selected (Fig 2) was a Veeder magnetic counter, form UM, operating on 110 volts direct current. This counter may be obtained wound for six volts and operated by a storage battery. It must operate on direct current.

It was desired to mark every cell after counting it, and to facilitate this the handle containing the "make and break" apparatus (contact stylus, Fig

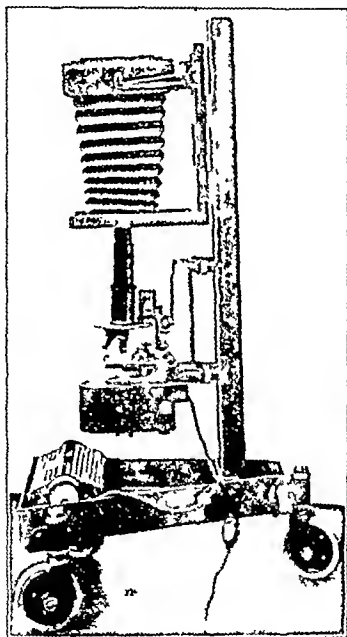


Fig 1—Photographic apparatus

3) was devised by the Mayo Clinic mechanic George Little. Mr Little's description of this very essential part of the apparatus is as follows:

"The fiber sleeve *A* is threaded at the ends to receive the terminals *G* and *H* and contains the bakelite plunger *B* and block *A*, *H* being threaded to receive the socket bushing *I*, through which passes cord *J*. The plunger *B* is limited in movement and retained in position by screw pin *D* in slot *C*. *F* is medium tone steel victrola needle driven into the plunger terminal *E*. *E* is pushed outward by the coil spring and, being threaded into the lower end of

plunger *B*, serves to keep *B* down against the screw pin *D*, and to keep current terminals, or switch points, *L*, *M* and *N* open. Point *L* is secured to one cord terminal as shown and *N* to the other. Bringing point *F* in contact with the paper and pushing lightly downward, causes sleeve *A* to carry block *K* down until contact is made with *L*, *M* and *N*, thus closing the circuit and causing the magnet to function and operate the counting-device."

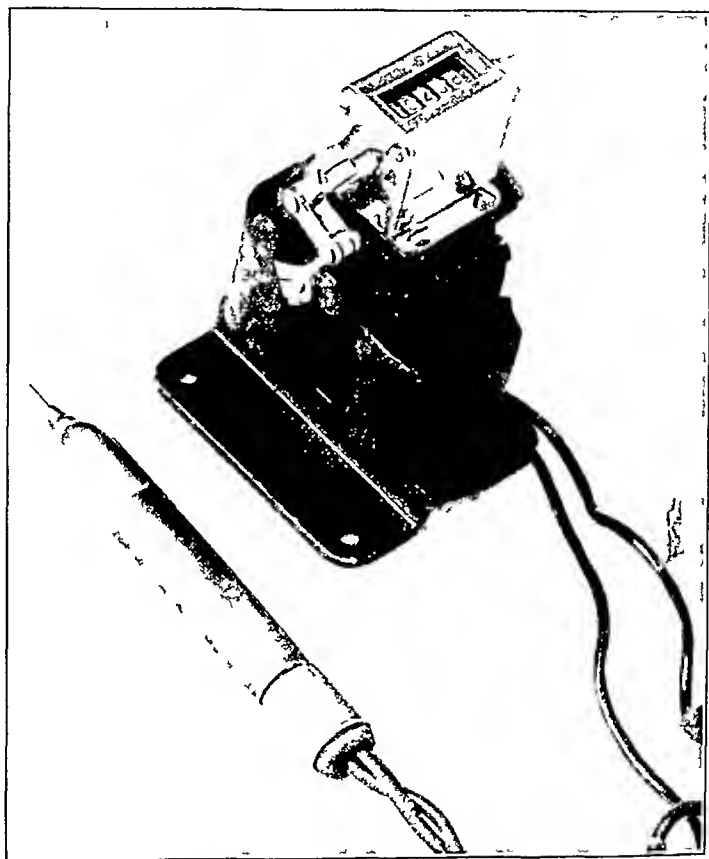


Fig 2—Automatic counter

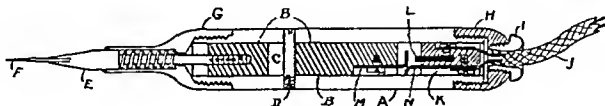


Fig 3—Contact stylus

Several series of counts have now been made with this apparatus. A typical count is illustrated in Fig 4. These counts have been compared with those made in the usual manner, and it appears that the average technician does not ordinarily count quite all of the cells in the field as registered on the photographic plate. This can be explained in various ways, but possibly the result of constant focusing that a microscopist practices in his effort to bring everything in the field into sharp focus, is that a few cells in the

field are missed by being thrown out of focus. The image of all the cells is registered on the photographic emulsion, however, even if they are not quite in focus.

While this method of counting erythrocytes is original as far as I am concerned, the idea is not new. The title of Amoys' paper on 'Experiments and Clinical Observations on the Hematinic Properties of Dialyzed Iron,' published nearly fifty years ago would not suggest the manner of making the blood counts, but the beautiful heliotype illustrations accompanying the paper (Fig. 5) excite immediate attention. The author says 'The individual or personal error of vision which is associated with all optical instruments is perhaps somewhat difficult to reduce to mathematic accuracy on account of the fact that constant observation fatigues the eyesight and

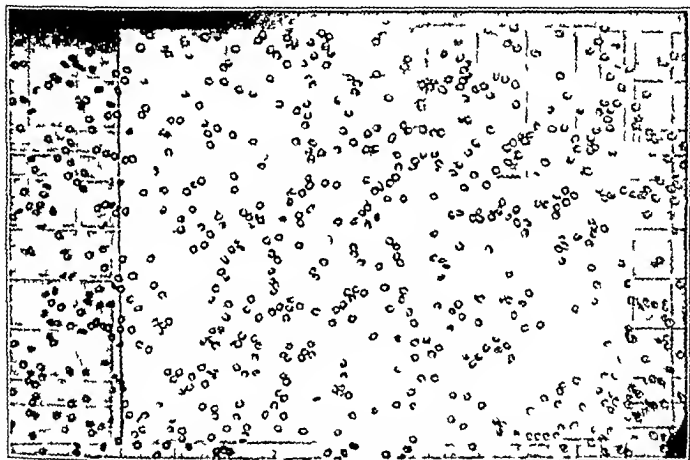


FIG. 4.—Erythrocyte count showing cells punched out in eighty squares

hence the results of a series of these observations are subject to an inconstant variation. In consequence of this apparent difficulty I decided to project upon a photographic plate the image of the corpuscles on the ruled slide, then to print from the negatives, and count upon the print the number of these corpuscles each one being obliterated as soon as counted.

Reference should also be made to a similar method used more recently by Haidesty³ in studying the number and arrangement of the fibers forming the spinal nerves of the frog. "In order to count a given section a photograph of it was fastened upon a small board of soft wood. An automatic registering machine, one common use of which is to count telegraph poles, was modified by attaching to its finger press a short steel rod. Into the end of this rod was inserted a needle." The fibers were punched out on the photograph with the needle and the count registered.

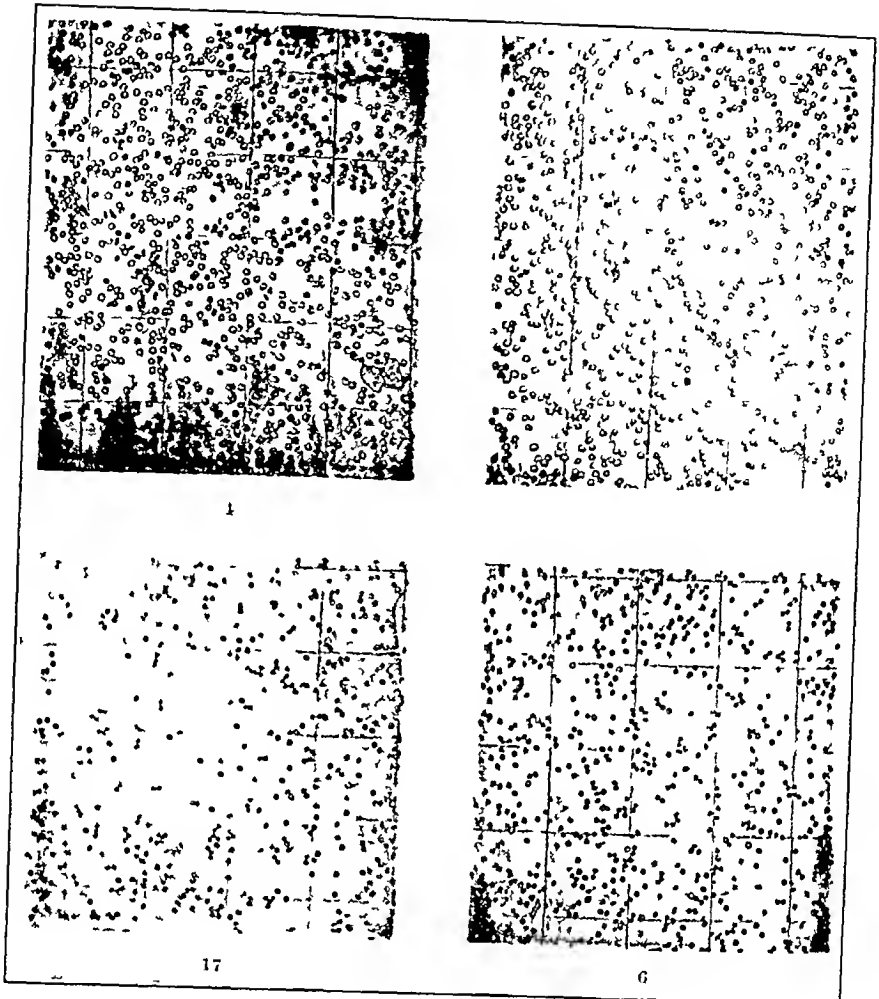


Fig 5—Copy of photograph by Amory

Thus fortified by the arguments of half a century ago, I offer anew a photographic method for counting blood cells

REFERENCES

- ¹Amory, Robert Experiments and Clinical Observations on the Hematinic Properties of Dialyzed Iron, Boston Med and Surg Jour, 1879, c, 454-459
- ²Gowers, W R On the Numeration of Blood Corpuscles, Lancet, 1877, ii, 797-798
- ³Hardesty, Irving The Number and Arrangement of the Fibers Forming the Spinal Nerves of the Frog (*Rana virescens*), Jour Comp Neurol, 1899, ix, 64-112
- ⁴Hayem and Nacet New Apparatus for Counting the Blood Corpuscles, London Med Rec, 1875, iii, 457
- ⁵Lyon, J F, and Thoma, R Ueber die Methode der Blutkörperzählung, Virchow's Arch f path Anat, 1881, lxxxiv, 131-154

DISCUSSION

Dr H J Corper—I have felt for a long time that we should be taking greater advantage of photographic methods in clinical pathology. I appreciate Dr Sanford's timely report and compliment him on it. I have been interested in photographic methods for some time and believe the time is coming when we will be urged to utilize photography wherever possible. There is a decided advantage in putting photographic copies in the clinical records.

as is now common with x ray pictures. Some day we will be putting into clinical records not only blood counts, but photographs of pathologic specimens as well. When you figure it up financially it is not expensive to use ordinary bromide paper. Direct photographs on bromide paper can go into the records at an average cost of a few cents per copy and are less susceptible of personal error.

Dr Wm G. Estlin—I was glad Dr Sanford brought up this method. We have been doing our blood counts by photography for about ten years using the little instrument called the euscope. You can get the whole four hundred squares. The last six or seven years we have been using ordinary bromide paper. I can say from our experience that the work does not cost as much and is certainly more accurate than the usual counts. With photographic methods you will be surprised with what little experience you can adopt them to your use.

Dr A H Sanford (closing)—I don't want you to get the impression that this is our routine method. Dr Cooper's suggestion is good. Some day this may be our routine. I am convinced, however, that for research purposes it is worth while and that is what we are using it for at present.

OCHRONOSIS*

By ERNEST SCOTT M D AND ROBERT A MOORE B A, COLUMBUS OHIO

VIRCHOW¹ in 1866 reported a postmortem on a sixty seven year old man, in whom he found that practically the entire cartilaginous system of the body was a coal black, 'als ob sie geradezu in gewöhnliche Tinte eingetaucht worden waren'. He called this condition 'ochronosis'. Virchow working with Kühne, after some chemical investigation, concluded that the pigment was a derivative of hemoglobin and probably was only an extreme degree of a similar pigmentation noted by him previously in old people, and especially inetics.

Since Virchow's original report there have been reported some fifty three cases of this disease. In the case about to be reported, the fifth, sixth and seventh costal cartilages of the right side were an intense black color. Despite the lack of external pigmentation and the finding of an apparently normal urine, there is evidence that this case is one of true ochronosis. Briefly this evidence is first the typical gross and microscopic appearance of ochronosis in the examined cartilages and second a pigment which chemically is iron free exhibits solubilities and staining reactions similar to the melanins is bleached by sunlight and gives no spectral absorption.

Case Report—R T fifty two years old white woman married was first admitted to Mt Carmel Hospital May 23 1922 on the service of Doctor C S Hamilton. Six months previously she had noticed a small lump in the right breast. She had not noticed any loss of weight or strength and had never suffered from any serious illness. There was no history of surgical operations. There had been no pregnancies and she passed the menopause four years previously. The patient denied the use of any drug containing phenol.

Physical Examination—The examination was essentially negative, except for a tumor about the size of a walnut in the central part of the right breast. The axillary nodes were

Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, at Dallas, Texas April 15 16 and 17 1926.
From the Department of Pathology, Ohio State University, Columbus Ohio.

not palpable. The blood pressure was 135/80. There was no abnormal pigmentation of any part of the body.

Laboratory Tests—Erythrocytes, 5,200,000, leucocytes, 5,200, polymorphonuclears, 35 per cent, small lymphocytes, 55 per cent, large lymphocytes, 10 per cent, eosinophiles, 0 per cent. The urine on numerous occasions showed a pyuria with a trace of albumin, but was not dark in color, nor did it turn dark on standing.

Course—The right breast was removed together with two thirds of the pectoralis major muscle and the axillary glands, May 24, 1922. It was noted at the operation that one axillary node was the size of a bean. Microscopic examination showed the tumor to be a scirrhous carcinoma. At the time of this operation no abnormality of the ribs was noted. The wound became infected and a sinus developed. From this, as well as from the blood, a pure culture of a hemolytic streptococcus was isolated. About July 1, 1922, the patient developed definite signs of an arthritis, involving particularly all the joints of the lower extremities. A month later the patient was discharged with an area the size of a dollar which was still draining and covered with granulation tissue. On October 3, 1922, she returned with a diffuse induration of the left breast, which was immediately relieved on curetting the sinus on the right chest wall, and the patient was again discharged. On January 11, 1923, she returned for a thorough curettage of the sinus. At this operation, the following operative notes were made, "Sinus is over the sixth rib in the nipple line, in the center of an area of red scar of half dollar size. In making a sufficient incision (eventually five inches outward from the parasternal line) it is found that the fifth, sixth and seventh costal cartilages are converted into a black substance, resembling coal. A portion of rib about one and one half inches long in the immediate neighborhood of the sinus is deprived of periosteum. There is no pus. The black ribs are removed." It was noted also that the costal cartilages above and below the black ones were not noticeably discolored. The patient at this time showed evidence of a definite arthritis of the larger joints. The sinus cleared up and the patient was discharged. A year later she developed symptoms of an obstructive jaundice and died February 9, 1924. A postmortem was not made. For the year following the finding of the black cartilages, the patient showed no abnormal pigmentation and the urine presented no abnormalities.

Pathologic Examination—Section of the costal cartilages showed the hyaline matrix to be of a diffuse yellow brown color without granular pigment while the cartilage cells here and there showed some amorphous granular pigmentation. The perichondrium was not pigmented.

Chemical Investigation—The pigment was soluble by boiling in 0.2 per cent NaOH and was soluble in cold strong acids. Microchemical reactions for iron were negative. Solutions of the pigment when placed in the spectroscope gave no absorption bands. Sections exposed to the sun were slowly bleached. Because of the small amount of material available, the actual chemical composition of the pigment could not be determined. The urine at no time showed a dark color and it did not turn dark on standing.

The etiology of ochronosis has been the subject of considerable debate, based both on observations on man and experimental animals. Based upon the association of certain urinary findings, we may conveniently divide the reported cases into four classes.

1. Vuchow¹ in reporting the first case felt that the condition was due to an imbibition of hemoglobin into the tissues. Nardi² reporting nine cases of ochronosis from Italy found that intraarticular injection of homogenous blood into rabbits caused the production of a black pigmentation of the joint cartilages.

2. Albrecht³ in 1902 reported a case with chemical investigation by Zdarek⁴ in which the condition was associated with alkaptonuria. He felt that the homogentisic acid united with the chondroitin sulphuric acid of the

cartilages giving the black pigment Gross and Allard reported a similar case with complete pathologic report by Landois⁸ and have subjected this theory to experimentation They found that if cartilage is placed in a neutral solution of homogentisic acid in the course of a month it will turn black and is indistinguishable histologically from true ochronosis

3 In 1906 Pick⁹ observed a case of ochronosis associated with the prolonged use of phenol He reviewed the literature up to that time and came to the conclusion that the condition was due to the deposition of a melanin derived from the oxidation of aromatic compounds such as phenol homogentisic acid etc Poulsen⁹ after an extensive study of nine cases with necropsy on two came to the same conclusion and proposed that the oxidation was carried out by the enzyme tyrosinase in the same manner formulated by von Furth and his coworkers for the animal melanos in general Beddard¹⁰ and later Beddard and Plumtree,¹¹ are very emphatic in their conception that the use of phenol by some of these cases is of more than passing importance, and cite the fact that one of their patients showed a decrease if not disappearance of the pigment, on discontinuing the use of phenol On the contrary, von Amstel⁷ feels that the use of phenol by these patients is only a coincidence but he does think that alkaptonuria and ochronosis bear some relation to each other Gross¹² has attempted to produce ochronosis by the daily injection of phenol for one year into a dog and calf They report entirely negative results Analysis of the time interval in the human cases between the initial use of phenol and the first appearance of the pigment shows a notable lack of accurate data, yet in a few cases we have figures of three years four years and one year

4 In addition to these well defined cases showing alkaptonuria or giving a history of the continued use of phenol there are certain cases as those of Hecker and Wolf¹⁴ and Poulsen's third case and the more recent case of Oppenheimer and Kline¹ with chemical investigation by Janner¹⁶ in which a definitely proved melanuria has been present

5 There are still other cases notably that of von Hanseemann¹ Haistson and Soltan¹⁸ and the present case in which the urine was apparently normal There are numerous cases including those of Virchow¹ Heide's two cases¹⁹ and others in which a urinary examination was not made

Most of the reported cases show a rather general pigmentation of the cartilaginous system of the body Yet there are several cases notably that of Albrecht³ and that of Ogden⁶ as commented on by Osler¹ in which the external pigmentation was limited to the ears The present case is of this type of localized ochronosis the pigment being localized as far as is known to the right costal cartilages particularly the fifth sixth and seventh

An analysis of the reported cases shows the disease to be about equally distributed in the two sexes and that it has occurred as early as twenty three years² and as late as eighty five years³ with an average of about fifty years An analysis of the associated diseases shows no one disease to be of such frequent occurrence as to be of etiologic significance yet one is impressed by the high incidence of chronic lesions of the joints and cardio-

vascular system Sodebergh²⁴ has expressed the opinion that all of these lesions are the result of the same cause as ochronosis, viz., a metabolic disorder, and not the cause per se. The fact that the present case presented the development of an arthritis and pigmentation of the costal cartilages simultaneously gives some support to this view.

In only one case do we find a thorough chemical investigation of the pure pigment, this work being reported by Janney¹⁶. He found on organic combustion analysis that the pigment has a constitution very similar to that of the melanins reported by Moirer. All authors agree that the pigment is spectroscopically inactive.

From this review it seems apparent, that the condition of ochronosis may occur with several associated conditions, to which has been attached an etiologic significance. The proof of the real significance of these associated conditions must be solved by the clinical pathologist, especially by a thorough examination of the urine and a chemical investigation of the pure pigment similar to the recently reported work of Janney.

SUMMARY

1. A case of ochronosis in which the known pigmentation occurred only in three of the right costal cartilages is reported.

2. The condition was associated with a chronic streptococcus infection and arthritis, subsequent to amputation of the breast for malignant disease. The relationship of the infection and ochronosis is problematic.

3. The views concerning the etiology of the condition are reviewed.

REFERENCES

- ¹Virehow, R. Ein Fall von Allgemeiner Ochronose der Knorpel und knorpelähnlichen Theile, Virehow's Arch f path Anat u Phys, 1866, *xxviii*, 212-219.
- ²Nardi, P. E. Sulla ochronosi delle cartilagini, Sperimentale, 1905, *lx*, 496-513.
- ³Albrecht. Über Ochronose, Ztschr f Heilk, 1902, *xxiii*, 366-378.
- ⁴Zdarek. Über den chemischen Befund bei Ochronose der Knorpel, *ibid*, 1900, *xxi*, 379-383.
- Gross und Allard. Untersuchungen über Alkaptonurie. Ztschr f klin Med, 1907, *64*. II 3 u 4.
- ⁶Landouzy, F. Zur Kenntniss der Ochronose. Virehow's Arch für path Anat u Phys, 1908, *cxviii*, 275-290.
- ⁷Gross und Allard. Experimenteller Beitrag zur Pathogenese der Ochronose. Arch f exper Path u Pharmacol, 1908, *lx*, 384-387.
- ⁸Pick. Über die Ochronose. Berl klin Wchnschr, 1906, *xlvi*, 478-482, 509-512, 556-560, 591-597.
- ⁹Poulsen. Über Ochronose bei Menschen und Thieren. Zeigler's Beitr, 1910, *xlvi*, 346-436, 437-498.
- ¹⁰Beddard, A. P. Ochronosis Associated with Carboluria. Quart Jour Med, 1909 *10*, *iii*, 329-336.
- ¹¹Beddard, A. P., and Plumtree, C. M. A Further Note on Ochronosis Associated with Carboluria. Quart Jour Med, 1912 *13*, *v*, 505-508.
- ¹²De Bruine Ploos von Amstel. Ochronose, Med Weekbl, 1909 *10*, *xxi*, 443-456.
- ¹³Gross. Über Ochronose, Deut med Wchnschr, 1910, *xlvi*, 368-369.
- ¹⁴Hecker und Wolf. Ein Fall von Ochronose, Festschr z Feier d 50 Jahr Besteh d Stadtkrankenhaus zu Dresden Friedrichstadt, 1899, 325-335.
- ¹⁵Oppenheimer, B. S. und Kline, B. S. Ochronosis, Arch Int Med, 1922, *xxviii*, 732-747.
- ¹⁶Janney. A Study of Ochronosis, Am Jour Med Sc, 1918, *elvi*, 59-68.
- ¹⁷Von Hansemann. Über Ochronose, Berl klin Wchnschr, 1892, *xxix*, 660-662.
- ¹⁸Hurstou und Soltan. A Case of Ochronosis, Brit Med Jour, 1908, 1230-1231.
- ¹⁹Heile. Ueber die Ochronose und die durch Formol verursachte pseudo ochronotische Färbung der Knorpel, Virehow's Arch für path Anat u Phys, 1900, *xl*, 148-173.

- ¹⁰Ogden Ein Fall von Alkaptonurie Ztschr f physiol Chem 1895 xx, 280 286
¹¹Osler Ochronosis The Pigmentation of Cartilages, Sclerotics, and Skin in Alkaptonuria, Lancet, 1904, i, 10 12
¹²Poulsen Ueber einem neuen Fall von Alkaptonurie mit Ochronosis, Munchen med Wehnschr, 1912, lx, 364 366
¹³Hunter W C Ochronosis Report of a Case with Associated Disease of the Adrenals, Ann Clin Med, 1925, iv, 250 260
¹⁴Soderbergh Ober otitis deformans ochronotica Neurol Centralbl, 1913, 1382 1393

CLINICAL RESULTS WITH PATHOGEN*

By OTTO LOWY M D NEWARK, N J †

VACCINE therapy had received several severe setbacks in the past because we did not utilize the fact that in order to be of value the vaccine used must consist of the identical organism which is causing the infection. While we have known that organisms morphologic and culturally are alike and yet different, we failed until recently to properly differentiate between the various strains of these bacteria.

It is an established fact that infections, particularly focal infections may give rise to symptoms in remote parts of the body. Here again we were confronted by our lack of properly identifying the offending organism. The work of Heist, Lacy, Solis Cohen and others have contributed largely toward clarifying some of our difficulties. Briefly stated. They have found that the whole coagulable blood of normal individuals contains immune bodies which prevent the growth of pathogenic organisms normally found on the various mucous membranes. They have also found that if an individual is deficient in immune bodies against certain bacteria, these bacteria grow luxuriantly in the individual's whole blood.

Burbank and Hadjopolis attack the problem from another angle. They make use of the well known complement fixation test, using as the antigen various strains of streptococci staphylococci gonococci. They also utilize the uative complement of the individual.

This method presents a number of technical difficulties as for instance the preparation and identification of the antigens furthermore there are a multiplicity of tests that have to be made in order to arrive at the proper classification. Burbank had been using twenty antigens. After two years work I have succeeded in preparing twelve antigens each antigen representing a different strain of streptococci.

When we turn to the clinical application of the two methods, we find that the pathogen method is by far the simpler and may even be considered as more accurate. Should however the source of infection be in locations such as the frontal sinus, the middle ear or some other inaccessible portion of the body, it would follow that the pathogen method could not be used, and in that event I believe we could fall back upon the complement fixation

Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas April 15 16 and 17 1926
 †Serologist to Newark Beth Israel Hospital.

Experimental—In order to determine what analogy if any, existed between the two methods, we examined five cases of chronic arthritis without deformity, by means of the pathogen method and found *Streptococcus viridans* in four cases and a nonhemolytic streptococcus in one case. These organisms were grown in pure culture. One portion of the growth was utilized for the preparation of an antigen and the other portion inoculated in the patient's own blood.

Results—Complement fixations were positive in varying degrees with all antigens. The pathogen showed growths in all instances. When the complement-fixation test was applied by using antigen one, against blood two, etc., we found that three of the strains gave positive reactions, which would seem to indicate that these strains were identical with one another.

*Treatment of Patients with Pathogen**—Ten cases of subacute and chronic arthritis, three cases of chronic bronchial asthma, were treated. Growths were obtained from either nose, throat, teeth and sputum. It may be noted that in the asthma cases plain broth cultures showed a large number of gram-positive and gram-negative organisms, whereas, using the whole blood only streptococci and pneumococci, were isolated. The vaccines were prepared and injections given at three-day intervals, starting with fifty million and increasing the dose gradually, going as high as one billion.

Reactions—Reactions with the exceptions of slight soreness at the site of injections were not observed.

Results—Four cases of subacute type of arthritis were completely relieved of their symptoms, two improved and four unimproved, of the asthma cases one improved, two unimproved.

Check Up—Up to date I have been able to check up only on two cases by again taking culture and attempting to grow them in the patient's own blood. Both of these were cases that had been improved and in both instances we were still able to demonstrate the streptococci in the growth.

CONCLUSIONS

1 Vaccine therapy in focal infections is of value only when the strain causing the infection is used for purpose of preparing vaccine.

2 Individuals suffering from focal infections should have the foci of infection removed.

3 Both the pathogen and complement-fixation tests are of value in determining the organisms.

DISCUSSION

Dr. George T. Caldwell—I am of the opinion that organisms do not grow in whole blood but are able to remain alive.

Dr. O. Lowy (closing)—I feel that any method which gives promise of being accurate and which gives results is worth while looking into.

*Clinical material obtained from the service of Dr. Szulip, Newark Beth Israel Hospital.

THE TREATMENT OF ONLY HUNDRED FIVE CASES OF ACID INTOXICATION WITH BUFFER SOLUTIONS*

By F. A. HECKER, M.D. OTTUMWA, IOWA

A REVIEW of the literature on acidosis or perhaps better stated acid intoxication at this time is extensive and the theories offered of the etiology are many and diverse. It is believed that acid intoxication is due to the accumulation of acid products in the blood which are due to faulty elimination which results in disturbed metabolism.

With our present knowledge acid intoxication may be divided into two groups.

Group 1. This group clinically is characterized by dry skin, dry tongue, air hunger and in the terminal stage by coma. The clinical symptoms it is believed are due to toxic substances which are the result of metabolic changes which deplete the tissues of their buffers.

Group 2. This group it is believed is due to toxic substances produced by the growth of bacteria in the tissues which they have invaded. By the absorption of the toxic substances an impaired chemical balance of the blood occurs. Consequently the vital organs and the tissues of the body are depleted in their buffers.

These two groups present chemical changes in the blood, the expired air, and the urine.

From the foregoing we are taught the blood of persons affected by acid intoxication shows a depletion of the blood buffers, namely the sodium bicarbonate and the disodium phosphate. In addition to the blood buffers the calcium, and the magnesium are also diminished in quantity when compared to the quantity normally present in the blood. When this chemical imbalance occurs it can be corrected by administering the needed salts by mouth or by introducing them intravenously.

The first method employed for the determination of acid intoxication was the carbon dioxide capacity of the plasma of Van Slyke and Cullen. This method was also employed for the determination of the progress of the treatment. As several specimens of blood were needed for the determination of the progress of the treatment we were confronted by objection by the relative of the patient. All of the patients treated were pay patients. Hence we were compelled to comply with the wish of the relative or discontinue the work. It is for this reason that this excellent method was discontinued.

The next method tried was the alveolar carbon dioxide tension method of Fidericia. Employing this method one must have the cooperation of the

Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 1, 16 and 17, 1936.
From the Laboratory of Clinical Pathology, St. Joseph Hospital, Ottumwa, Ia.

patient This at times is difficult if not impossible to obtain if the patient is in a semicomatose condition or is desperately ill Hence this method was discontinued

The next method tried was the determination of the P_H of the urine with the hydrogen-ion apparatus The specimen of urine was collected voluntarily or with the catheter All specimens of urine collected were sent to the laboratory in chemically clean, tightly stoppered bottles and were stored in the ice chest until titrated With this method the objections offered in the two foregoing methods were overcome and was the method selected for the determination of acid intoxication and the progress of the treatment

The Michaelis Fraction—The observed H-ion concentration of the urine

$$\frac{\text{Primary Phosphates}}{\text{Secondary Phosphates}} = \frac{H}{2 \times 10^{-7}}$$

$$\begin{array}{l} 2 \times 10^{-8} \\ 2 \times 10^{-7} \\ 2 \times 10^{-6} \\ 2 \times 10^{-5} \end{array}$$

I considered a urine having a P_H of 5.00 or less as indicative of acid intoxication

The following glassware is employed An all-glass still, a two-way glass stopcock, an all glass twenty c.c. syringe, the barrel of a one c.c. syringe which has been pulled down to one-fourth of an inch in diameter and three, five hundred c.c. Erlenmeyer flasks All glassware and rubber tubing must be chemically clean

A good method for the distillation of water is as follows 800 c.c. water is poured into the flask of the still, after which a few crystals of potassium permanganate are added and the distillation is begun The first 100 c.c. water coming over is collected in one of the Erlenmeyer flasks and used for rinsing them One of the flasks is placed under the outlet of the still and the remaining two flasks are stoppered with gauze As soon as 500 c.c. water has been collected it is equally divided in the stoppered flasks To one of the flasks is added 4.25 gm. of sodium chloride C.P.

The two flasks containing the water and the stopcock and accessories wrapped in a clean towel are now placed in the autoclave and sterilized When sterilization has been completed the flasks are removed from the autoclave and one of them is cooled in running water until it is comfortably tolerated by the back of the hand This step is followed by the determination of the dose of the alkali

The quantity of the dose of the alkali is determined as follows at this time One-third to one-fourth of the weight in grams per kilogram of body weight in the proportion of two parts of sodium bicarbonate to one part of disodium phosphate minus 4.25 grams of the sodium chloride The weighed amounts of the sodium bicarbonate and the sodium phosphate are then dissolved in the cooled flask The contents of the other flask, one-half of the sterile water, is then added to the flask in which the salts have been dissolved A 10 c.c. sample of this solution is placed in a chemically clean beaker and is titrated as follows

The burette is filled with a 10 per cent solution of the monopotassium phosphate. The beaker is placed on the stand of the hydrogen ion apparatus. The electrodes are immersed in the solution and the hydrogen electrode charged with hydrogen gas. This step is followed by allowing the solution of monopotassium phosphate to drop into the solution in the beaker. The solution in the beaker is stirred gently while the monopotassium phosphate is dropping into it. After 8 c.c. of the solution in the burette has dropped into the beaker the stopcock of the burette is turned off and a trial titer is made with the hydrogen ion apparatus. If the bridge reading does not correspond to the P_H of 7.00 the solution in the burette is added and trial titers are made from

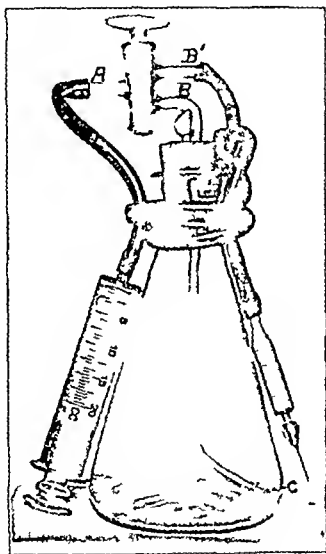


Fig. 1—Assembled apparatus used for giving intravenous buffer solution.

time to time. The moment the bridge reading approaches a P_H of 7.00 the burette stopcock is turned off and a routine titer is made. If the reading is not correct continue the foregoing technique until the desired P_H of 7.00 is attained. This step is followed by reading the burette from which the volumetric correction can be determined and the 500 c.c. of the solution is buffered with monopotassium phosphate to a P_H of 7.00. The solution is now filtered through sterile cotton.

The stopper supporting the two way stopcock is pressed firmly into the mouth of the flask containing the buffer solution. The outlet A when the valve of the stopcock is set at B communicates with the glass tubing C which

is immersed in the solution in the flask. The outlet A' when the valve is set at B' communicates with the syringe attached to the rubber tubing attached to the outlet A. Hence to operate the apparatus one turns the valve of the stopcock to the position A-B-C to fill the syringe and to the position A'-B' A to discharge the content of the syringe through the hypodermic needle attached to the glass connection. All air must be removed from the rubber tubing and the glass connection. The flask is now set aside and the blood pressure, pulse, and fever taken. This step is followed by the routine preparation of the site for the intravenous puncture and the intravenous puncture is made. The moment the needle enters the vein, blood appears in the glass connection to which the needle is attached. The next step is that of making gentle pressure with the syringe and if there is no distention of the overlying tissue at which the intravenous puncture has been made the intravenous administration of the solution is begun. The usual time required to give the dose is fifteen to twenty minutes.

The reaction following the intravenous administration of the buffer solution varies. The peak of the alkalinity of the urine varies in time. The chill varies in time, onset, and duration. The severity of the chill varies from a feeling of chilliness to a severe chill. The respiration as a rule is not affected, although in several instances a partial cessation of respiration has occurred for a short time. The fever usually rises rapidly sometimes reaching 107° F. Delirium and restlessness of varying severity sometimes follow the intravenous administration of the dose. In most instances the patient becomes quiet. The sweating usually follows the peak of the fever and the chill.

Immediately after the buffer has been given the patient should be covered with a woolen blanket and then packed in a hot pack to promote sweating, and usually kept for an hour. There are cases however when the hot pack should be continued as a matter of observation following the reaction. The patient should be well covered with a woolen blanket when the hot pack is removed and the wet gown should be replaced with a dry one. At the end of the second hour the blanket is removed and the patient is covered with routine bedding. The rectal temperature should be taken at half-hour intervals for two to four hours after the dose has been given, and then taken every two hours for the remaining twenty-four hours. Many times the recipient of the buffer solution is very thirsty and should be given water freely unless otherwise indicated.

The report offered in this paper was begun in 1923 and since that time 105 cases of all kinds have been treated with the buffered solutions to determine if possible their value in all affections which are accompanied by or followed by acid intoxication. All of the patients treated with the buffered solutions were desperately ill. It is not the purpose of the writer to lead the reader to believe that a panacea for acid intoxication has been found. But the writer does believe that many cases have been helped over the rough spots with the aid of the buffered solutions. The following tabulation shows the success and the failure of treated cases. Of 105 cases treated, 65 recovered and 36 died, 4 showed little or no improvement from the treatment but recovered.

CASES TREATED WITH BUFFERED SOLUTIONS

Diabetes			Nephritis all types		
Recoveries	-----	4	Recoveries	-----	9
Deaths	-----	0	Deaths	-----	0
Erysipelas			Hystorectomy		
Recoveries	-----	6	Recoveries	-----	1
Deaths	-----	1	Deaths	-----	1
Eclampsia			Cholecystotomy		
Recoveries	-----	4	Recoveries	-----	2
Deaths	-----	2	Deaths	-----	1
Pneumonia			Cholecystectomy		
Recoveries	-----	4	Recoveries	-----	1
Deaths	-----	0	Deaths	-----	1
Pyelitis			One case vomiting of pregnancy (not im- proved)		
Recoveries	-----	4	One case pyonephrosis during pregnancy (not improved)		
Improvement	-----	1	One case streptococcus empyema with pyo- nephrosis (some improvement)		
Streptococcus peritonitis			One case streptococcus septicaemia (recov- ered)		
Recoveries	-----	0	One case pelvic abscess following delivery pre and postoperative treatment (recov- ered)		
Deaths	-----	3	One case ruptured gall bladder preoperative (recovered)		
Postoperative appendectomy			Toxic exophthalmic goiter preoperative (re- covered)		
Recoveries	-----	0	One case scrotal abscess postoperative (died)		
Deaths	-----	4	One case ruptured pancreas postoperative (recovered)		
Puerperal sepsis			One case prostatectomy postoperative (re- covered)		
Recoveries	-----	3	One case suprapubic drainage postoperative (died)		
Deaths	-----	1	One case ruptured tube postoperative (re- covered)		
Puerperal sapremia					
Recoveries	-----	4			
Deaths	-----	0			
Streptococcus septicaemia with endocarditis					
Improved (died later)	-----	1			
No improvement	-----	0			
Streptococcus septicaemia following abortion					
Recoveries	-----	0			
Deaths	-----	4			
Streptococcus sore throat					
Recoveries	-----	4			
Deaths	-----	0			

Before closing I wish to thank the physicians who so kindly permitted me to give buffer solutions to their patients. I also wish to thank Dr. A. Itano formerly of the Massachusetts Agricultural College for the many suggestions when this work was begun.

SUMMARY

Thus far no ill effects have followed the intravenous administration of the buffer solutions.

The buffer solutions are not a panacea for the treatment of acid intoxication. Many times hopeful cases terminate in failure.

Theoretically the buffer solutions as a means for replacing the depleted buffers is correct, provided our present teaching is correct. Namely, that the blood buffers are the sodium bicarbonate and the disodium phosphate.

The titration of the urine with the hydrogen-ion apparatus is not difficult, and is a good method for the determination of acid intoxication and the progress of the treatment. The determination of the dose at this time is not difficult. Making the buffer solution and its administration is simple and quickly performed.

DIABETIC GANGRENE TREATED BY INSULIN*

BY HORACE GRAY, M.D., SANTA BARBARA, CALIF.

UNDER this title DuPié¹ has reported an instance of moist gangrene with ulcer exposing the tendons, "soundly healed" after three months. A similar satisfactory outcome seems worth putting on record, in view of the outright preference for operation on the part of medical men of experience with diabetes. Joslin² for example wrote "One hears of so few recoveries with enjoyment of life after months of medical treatment that I cannot help urging surgery at an early stage", and Blotner and Fitz "have not been particularly struck by the effect of insulin upon the healing of gangrene once it has developed."³

REPORT OF CASE

Mrs. E. J. A., a negro woman, aged sixty-six years and eleven months, 156.2 cm (61.5 inches) tall, began gradually to be troubled with nocturia and pruritus vulvae about July 1, 1918. Six months later she complained to a doctor, who then found sugar in the urine. Her weight had been greatest in 1914, namely 200 pounds with her clothes, while at the time of diagnosis it was about 185 pounds, and was 167 when last noted, one month before she came to me. This visit was the result of neglect of treatment (other than temporary abstinence from free sugar for a short while after diagnosis), eventuating in the following complaints: "Six weeks ago a gummy discharge on her stocking, then four weeks ago the ball of the right big toe 'peeled off', but still she wore her shoe and did the work in her own little house alone. Suddenly six days ago she found her foot discolored, especially on top, when she took off her shoes at 10:30 P.M." The next day, January 4, she stayed up around the house, shoeless. Jan. 5 and 6 she spent abed, finally calling a doctor in the evening. A sample of urine contained 20 per cent of sugar but no diacetic acid. On Jan. 9, when I first saw her, a specimen contained 27 per cent sugar, but no diacetic, then not later. Her temperature was 100.4°, pulse 112, respiration 20, blood pressure 145/85, white blood count, 24,600. Her right foot was all bluish red, and under the big toe was a sinus oozing only a little pus, while on the dorsum was an ulcer 2.5 cm wide, foul but seminspissated. The lung and other examinations were negative. The Wassermann and blood culture turned out negative. In the office she was given 10 units of insulin and sent into hospital at 5 P.M., where the urine was obtained at intervals of one to two hours, and after each positive test, ten units of insulin were administered subcutaneously. At 5 A.M. the voiding was sugar free, insulin totaling 50 units. Weight 147 pounds net (66.7 kg). The insulin was cut to 10 units three times a day a.c., and a diet was given of about 0.5 gm. protein and 15 calories per kilo, which was taught to her on the basis of Joslin's maintenance diet C 7 PF 5, (about carbohydrate 70 grams, protein 35, fat 60, calories 1000). No twenty-four hour urine thereafter contained more than 13 grams of sugar, and since January 16, the sixth day in hospital, sugar was found only once in the remaining 35 days in hospital and not at all in any of the

*From the Santa Barbara Clinic.

24 hour collections submitted at intervals since, including the latest on Aug 9. The insulin was gradually reduced to zero twenty days after admission, while still on 1000 calories. The diet was then gradually increased to C 135, I 60, F 90, Cal 1630, on which he was discharged February 22, 1926.

The foot was of course shown to a surgeon at her first visit, and she was watched carefully with a view to probable operation, especially as there was some pain in the foot, which Joslin regards as an indication for operation. X-ray examination by Dr. I. G. Ware showed marked bony absorption and decalcification of the distal phalanges of the right toes especially of the big toe, also of the distal portion of the proximal phalanx of the big toe, and lesser changes in the toes of the left foot. The treatment at first consisted of hot and cold plunges for ten minutes three times a day (the cold had to be omitted because it increased the pain) followed by dry heat with an electric baker, wet corrosive dressing and elevation of the foot for thirty minutes. Alternate hanging of the foot over the edge of the bed, as in Bierer's exercise cycle, was discontinued because painful. Diarrhea became free but as the fever decreased only in part the order was changed on January 15 to dry dressings. The next day the temperature fell to normal and remained so. The foot improved very gradually but there was no setback so that on February 22 she was allowed to go home though neither ulcer nor sinus was completely healed. Total healing occurred about May 9. Follow up August 9 seven months after I first saw her revealed body weight 150 pounds dressed the twenty four amount sugar free, the foot sinus and ulcer both healed and indeed the second and middle toes capable of a moderate amount of motion dorsally. The patient was found caring not only for herself but for house guests attending a church convention.

REFERENCES

- ¹DuPré W. H. Diabetic Gangrene treated by Insulin. *British Med. Jour.*, July 5 1924, ii 12.
- ²Joslin, E. P. *Diabetes*. Philadelphia 1921, ed. 3, p. 641.
- ³Blotner, H., and Fitz R. On Diabetic Gangrene. *Boston Med. and Surg. Jour.*, June 24 1926, cxiv 1158.

LABORATORY METHODS

THE STABILITY OF CARBOHYDRATE MEDIUMS*

By LUCY DELL HENRY, B Sc, AND M S MARSHALL, PH D, LANSING, MICH

THE identification of bacterial species plays an important rôle in the daily routine of a diagnostic laboratory. The means of identification must be unequivocal. When discrepancies occur two possibilities may be considered in locating their source,—the technic (including bacteriologic reagents), and the organism, for no living organism is invariant. For example, if an organism presents many of the ordinary characteristics of a certain species, but its reaction to a certain carbohydrate is eccentric for that species, the carbohydrate medium may be at fault or the organism may be peculiar in this respect. With the primary purpose of determining the conditions under which our carbohydrate mediums were to be relied upon, a series of experiments was made over an extended period. In some respects the work, directed as it was toward practical ends rather than toward fundamental scientific investigation, is obviously superficial. However, the results, briefly stated, may be at least of practical value to other workers as they are to us.

The use of filtration as a means of sterilizing carbohydrate solutions for bacteriologic work, although in all cases not absolutely necessary, is certainly from the standpoint of the integrity of the sugar the only safe procedure. Carbohydrates heated in culture mediums not always neutral in reaction are very likely to undergo some hydrolysis, sometimes to an objectionable degree. This premise has been accepted on the basis of the recommendations of Kendall and his associates¹ pertinent to work in biologic methods of identifying carbohydrates, on the basis of the discrepancies occurring in the literature regarding fermentation reactions, and on the basis of our own experiences with difficulties for which no explanation other than heating of the carbohydrates could be found. There certainly exist occasional variations in the fermentation reactions of some well-defined bacterial species, but one has every right to expect a reasonable degree of stability under comparable conditions.

EXPERIMENTAL

Using seven of the more frequently used carbohydrates, dextrose, lactose, sucrose, levulose, maltose, mannite, and xylose, 20 per cent aqueous solutions of each were prepared and filtered through sterile Mandler filters. These were stored in Pyrex glassware at 5° C during the experimental period. The medium in which these solutions were tested consisted of beef extract broth,

*From the Bureau of Laboratories Michigan Department of Health
Received for publication September 1 1926

made sugar free by incubation with *L. coli communior* and containing the usual 10 per cent peptone and 0.5 per cent NaCl, with 0.5 per cent agar and Andrade's indicator, the final product having a P_H of 7.1. The carbohydrate concentration was made 0.5 per cent in the melted partially cooled agar. This gives a semisolid base into which inoculations may be made by straight stabs in the center of the tubes. Thirteen cultures were used for checking all members of the euterie group, and through long culture on artificial medium presumably stabilized *B. coli communior*, *L. coli communis*, *B. typhosus* (two strains), *B. paratyphosus A*, *B. paratyphosus B*, *B. dysenteriae* (Shiga, Flexner Mt. Desert, and Hiss 1 types), Morgan's bacillus *B. aerogenes*, and *B. alkaligenes*. During the experimental period these cultures were transferred weekly on beef infusion agar slants.

For a period of one year each carbohydrate was tested weekly in freshly made medium against each of the above cultures. Thereafter the tests were made at uneven intervals up to a period of twenty months from the time of the original filtration of the sugars. At the final testing a duplicate set of sugar mediums was prepared from freshly made 20 per cent sugar solutions, and brom thymol blue was used instead of Andrade's indicator in order more closely to follow and compare the P_H changes. Without attempting to illustrate by the extensive tables necessary to show the details of the reactions of the series, the results may be briefly discussed.

Dextrose is fermented probably by all organisms capable of fermenting any carbohydrate and inasmuch as Kendall has shown that very minute amounts of sugar may be demonstrated by biologic tests a considerable decomposition might have occurred with dextrose without destroying its apparent specificity as a fermentable sugar. Hence it is not surprising to find that the extended period of storage of the dextrose solution shows no effect as biologically tested. The *B. coli* strains *B. aerogenes* Morgan's bacillus, and the paratyphoid cultures produced acid and gas and the other cultures excepting, of course, *B. alkaligenes* produced acid both at the beginning and at the end of the twenty months period.

Lactose, with which more trouble might be expected, was very consistent during the entire period with the exception of doubtful fermentations on several occasions with Morgan's bacillus and with the four dysentery strains. These reactions are explained by the fact that the sugar medium, made up on scheduled time, was not inoculated until some days later. The sugar solution per se was stable, but the sugar medium, although stored at the same temperature, was not. This has been confirmed by repeated observations not only with lactose but with other carbohydrates.

Sucrose solution remained stable during the twenty month period. Several abnormal reactions appeared exactly as in the lactose tests and on the same dates due to storage of the medium containing the sugar. The Flexner dysentery strain produced acid from the sucrose solution for the first twenty seven weeks, and thereafter produced no acid with the exception above noted. In our final test, however, in which freshly prepared sucrose solution as well as the stored one was used, this culture produced alkali in twenty four hours.

in thirty-one hours acid production had reduced the reaction slightly (P_H 6.8), and in forty-eight hours the acid production was as marked as with any sucrose fermenting culture. Thus time is an important factor in following carbohydrate fermentations. The progressive reactions in the two sets of mediums were, however, exactly parallel, the use of brom thymol blue making possible a detailed record of changes in P_H value.

Maltose offered more difficulties than any other carbohydrate used, although its stability stored in 20 per cent solution was constant so far as biologic tests were concerned. The *B. coli* strains and *B. aerogenes*, the two typhoid strains, the *B. paratyphosus* A strain, and the Flexner and Hiss Y dysentery strains showed a constant reaction during the twenty-month period. Beginning on the seventh week the *B. paratyphosus* B strain, normally producing acid and gas, failed for four consecutive weeks to react. Using fresh carbohydrate solution, three strains originating from the same source were tested against this sugar, one the experimental culture, one which had been kept on similar medium but less frequently transferred, and one which had been kept on a sugar-free semisolid medium. Of these the first gave no fermentation (the same response as given to the stored maltose solution), the second gave some, and the third gave normal acid and gas production. By putting the experimental strain through a generation on sugar-free semisolid agar, the normal fermentation reaction returned, and never again disappeared. The Shiga dysentery strain gave several peculiar reactions but these appear to be due to the period of incubation of the inoculated tubes. In the final experiment, comparing old with fresh sugar solutions, the results with the Shiga culture were exactly parallel: slight acid in two and one-half hours, definite in four and one-half hours, neutral again from eight to thirty hours, after which there was a very slight trace of acid. The Mt. Desert dysentery culture and Morgan's bacillus gave similar reactions.

Levulose is normally fermented by all cultures of those used except *B. alkaligenes* and showed no change during the twenty months of biologic testing.

Mannite was stable throughout the twenty months. *Xylose* showed no change over a period of twelve months, at which time the supply of filtered sugar was exhausted.

The specific rotation of the sugar solutions was measured after one month, after two months, and after twenty months. The results are not all that might be desired, since the sugars were not specially purified, and storage was under practical rather than under ideal conditions, thus the physical conditions for a high degree of accuracy were not met. The goal was not so much the determination of specific rotation as the securing of a control for our biologic results. With fresh solutions there was no great discrepancy between the rotation of the sugar solutions as measured and tabulated values² secured under optimum conditions. After twenty months of storage, however, the specific rotation was from 10 to 40 per cent greater in all of the carbohydrate solutions except mannite and xylose. Mannite does not rotate the plane of polarized light, and did not rotate it after twenty months standing. Xylose

could not be tested because of insufficient amount. Determination of the concentration of sugar in several of the stored solutions showed them to be less than 22 per cent, an increase of less than 2 per cent due to evaporation, so that the considerable increase in rotation could not be explained on this basis.

SUMMARY

Twenty per cent unheated filtered solutions of dextrose, lactose, maltose, sucrose, mannite, and levulose stored at 5° C. appear to retain their specific properties with regard to biologic fermentation for a period of at least twenty months, xylose similarly prepared is stable for at least twelve months. The addition of such solutions to culture medium aseptically to avoid heating specifically a beef extract sugar free semisolid agar furnishes an excellent means of checking fermentation characteristics of bacteria but the storage of the medium after the addition of the carbohydrate for more than a few days renders the fermentation reactions nonspecific. Variation in the fermentation of some carbohydrates by some bacterial strains may under unknown circumstances occur, but every possible means of demonstrating that such variation does not occur should be exhausted before accepting such a conclusion. Fermentation reactions should be read for example at four, six, eight, twenty-four, and forty-eight hours from the time of inoculation for it is essential that one view fermentation as a progressive process with several possibilities rather than as a static definitely positive or negative matter.

REFERENCES

- ¹Kendall and Yoshida. Jour. Infect. Dis. 1923, XLIII 305
 Kendall. Ibid. 1923 XLIII 362
 Kendall and Yoshida. Ibid. 1923 XLIII 69
 Kendall, Bly and Haner. Ibid. 1923 XLIII 377
²Landolt. The Optical Rotating Power of Organic Substances and Its Practical Applications
 1902 Easton, Pa. Chemical Pub. Co.
 Browne. A Handbook of Sugar Analysis 1912
 Mackenzie. The Sugars and Their Simple Derivatives 1913
 Armstrong. The Simple Carbohydrates and the Glucosides 1919 Chicago Ill. Chicago Med. Book Co.

A MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS* (SECOND COMMUNICATION)

By B. S. KLINE, M.D., AND A. M. YOUNG, M.D. CLEVELAND, OHIO

IN A previous paper¹ details of a microscopic slide precipitation test for syphilis with Kahn's antigen dilution were given. In a report (to be published) the results obtained by this method in 2809 tests will be given in detail. In that study it was found that there was agreement of the slide precipitation test with the condition of the patient in 94.9 per cent. The Kahn test and the Wassermann test with an ether insoluble antigen agreed with the condition of the patient in 95.2 per cent. The Wassermann test with an acetone insoluble antigen agreed with the condition of the patient in 93.5 per cent.

Although the agreement of the slide precipitation test with the condition of the patient was practically the same in number as that of the Kahn test and a sensitive Wassermann test, its disagreement (in 5 of 100 tests) was more frequently on the basis of a positive reaction in nonlucetic serum, whereas the disagreement of the other tests was more frequently due to a negative reaction in luetic serum. It became apparent therefore that the slide precipitation test as performed, although even more sensitive than the Wassermann and the Kahn tests, did not quite equal those tests in specificity. This difficulty has been thoroughly overcome by the use of an antigen completely free of precipitate at room temperature, by shortening the time of the test and especially by more thorough immediate mixture of the antigen dilution and serum. The vast majority of the 2809 tests were done with an antigen containing a fine precipitate at room temperature, dissolved just before use by placing the ampule in hot water. The immediate slide precipitation test as now performed is not only more sensitive than the Wassermann test but just as specific (see Tables I and II). Furthermore, it requires no humidifier cover and may be done regardless of the humidity of the room. The temperature, however, should be no less than 70° F and the glassware and ingredients should not be cold. In addition, the test has been simplified by the use of a microscopic slide 2 by 3 inches holding 12 paraffin rings instead of 3 microscopic slides, 1 by 3 inches, each holding 4 rings.

TABLE I

IMMEDIATE SLIDE PRECIPITATION AND WASSERMANN TESTS WITH CLINICAL COMPARISON

52 Luetic 3 Doubtful 316 Nonluetie sera	Immediate Slide Precipitation Test (Antigen clear at room temperature)		Wassermann Test (Ether insoluble antigen)	
	Tests	%	Tests	%
Agreement	395	98.5	368	97.35
Disagreement				
a One test positive, patient nonluetie	2	0.5	3	0.8
b One test negative, patient luetie	4	1.0	7	1.85
	6	1.5	10	2.65
Total	401		378	

TABLE II

COMPARISON OF IMMEDIATE SLIDE PRECIPITATION AND WASSERMANN TESTS

	MT SINAI HOSPITAL		CLEVELAND HEALTH DEPARTMENT KINDNESS OF MR. C. REINSTEIN	
	Tests	%	Tests	%
Agreement	364	92.62	409	91.7
Relative Agreement	21	5.34	23	5.6
Agreement or Relative Agreement	385	97.96	432	97.3
Disagreement	8	2.04	14	2.7
Total	393		446	
Wassermann Anticomplementary, Slide Precipitation Test Variable	20	4.5	7	1.5
	413 Done		453 Done	

866 Slide Precipitation Tests in All

Evaluation according to the method of Kahn —

Positive Reaction ++++ + and ++

Doubtful Reaction + and ±

Agreement = Positive or negative by both methods

Relative Agreement = Positive or negative by one method and doubtful with the other

IMMEDIATE SLIDE PRECIPITATION TEST FOR SYPHILIS

Glassware—Microscopic slides 2 by 3 inches as purchased are rubbed on both sides with bon ami paste (prepared by allowing a cake of bon ami to remain in sufficient warm water to cover it for twelve hours or more). As soon as the paste is dry (in about five minutes) it is completely removed from the slide with a soft muslin cloth. For convenience the slides covered with paste may be stuck to each other, allowed to dry and cleaned at any time. Upon the clean slides, 12 paraffin rings each with an inside diameter of 11 to 12 mm, are

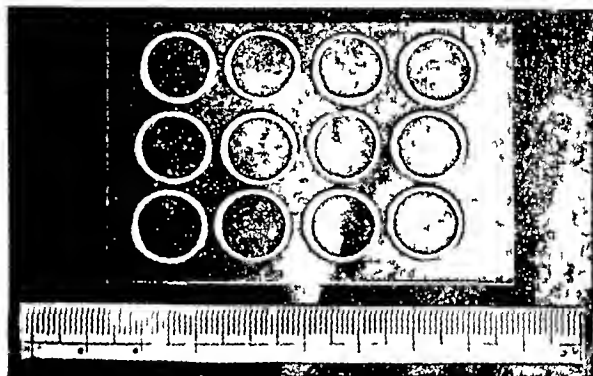


Fig 1—Microscopic slide 2 by 3 inches holding twelve paraffin rings (exact size)

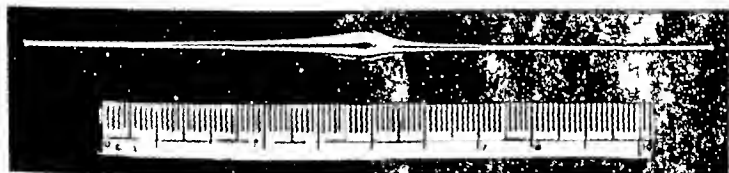


Fig —Capillary pipette for antigen dilution (exact size)

mounted (see Fig 1). After use the slides may be washed in hot water and prepared again as outlined above.

Instrument for Making Paraffin Rings—This is essentially the instrument proposed by Green.⁴ A piece of soft iron wire (No 28) 14 cm in length is wound twice tightly about a test tube $12\frac{1}{2}$ to 13 mm in outside diameter forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch of the free end. After removing the looped wire from the test tube a piece of linen thread (No 12) about 1 yard long is started from the free end of the shaft after being fastened here by a single twist of the two free ends. Three long

turns are made reaching the loop which is then tightly wound with the thread, the winding is continued up the shaft to the free end where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft. It is then reshaped by working the loop against the bottom of the test tube mentioned above. The shaft is then inserted into the handle of a teasing needle or into a straight hemostatic forceps.

The paraffin rings are made by dipping the instrument into smoking paraffin (about 120°C), drawing quickly at one point and transferring the remainder to the glass slide.

Pipettes—The pipettes needed for delivering the sera are the ordinary 1 c c pipettes graduated in 0.01 c c. The pipette for the antigen dilution is a capillary pipette made from glass tubing 6 to 8 mm in diameter (see Fig 2) with the tube about $\frac{1}{2}$ mm in diameter, delivering a drop equal to 0.0075 to 0.0085 c c.

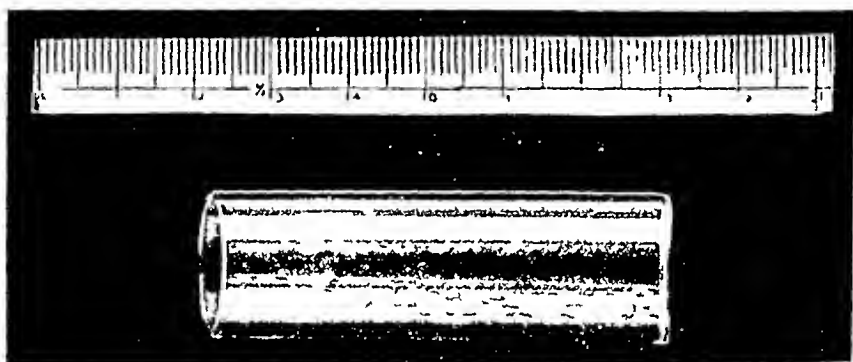


Fig 3—Vial for antigen dilution (exact size)

Vials—Vials for preparing the antigen dilution similar to those recommended by Kahn are satisfactory although those of a somewhat larger size (6 cm in length, 2 cm in outside diameter) are preferable. (See Fig 3)

Antigen—The antigen (an ether insoluble alcoholic extract of beef heart powder) and the antigen dilution are prepared as for the Kahn test. It is important that the antigen contains no precipitate at room temperature. If after cholesterolization and filtration of the antigen a precipitate forms at room temperature, this should be removed by placing the antigen on ice for an hour and then filtering it through filter paper. In the future it is proposed to place the cholesterolized antigen on ice for an hour before filtering it.

Antigen Dilution—The antigen dilution should be made up just before pipetting the sera. Some antigen dilutions have been found to work only within fifteen minutes of their preparation. An average antigen dilution may still be used forty-five minutes after its preparation. Some antigens work best beginning about ten minutes after their preparation, others work well beginning immediately after their preparation. The action of the antigen dilution in the slide test has been found unsatisfactory at low temperatures. Accordingly it is important that the room temperature be no less

than 70° F and that the glassware and ingredients be not cold. A blotter or piece of felt on the table top upon which the tests are set up is advisable.

Sera—These are obtained as for the Wasserman test, care being exercised that they contain no red blood cells or foreign material. Before use, they are heated to 56° C for one half hour.

THE IMMEDIATE SLIDE PRECIPITATION TEST FOR SYPHILIS

Into each of the twelve rings on the slide 0.05 cc to 0.06 cc of the undiluted serum to be tested is delivered from a pipette. The tip of the pipette is placed in the center of the ring and the serum allowed to run out. After all the sera are pipetted one drop of the antigen dilution (0.0075 to 0.0085 cc) is allowed to fall from the capillary pipette into the serum in each ring. After all the antigen is pipetted the small amount in each ring is evenly distributed by stirring the mixture with a toothpick (a new toothpick with flat end a few mm in width is used for each test). After the twelve mixtures have been made the slide is rocked and rotated between the thumbs and index fingers of both hands for two to three minutes (depending upon the character of the antigen dilution) and read immediately. To insure even movement of the slide both wrists and hands should participate equally. The slide should not be fixed or relatively fixed by one hand and moved by the other. Any spilling from a chamber makes the reaction therein unsatisfactory and the serum concerned should be retested. The readings are made through the microscope (16 mm objective 10 or 12.5 eyepiece) with the light cut down as in studying urinary sediments and recorded in terms of phases according to the degree of clumping and the size of the clumps. Because of their importance, it is strongly recommended that all the tests be done in duplicate, using different antigens.

COMMENT

The microscopic slide precipitation test for syphilis has been further simplified and improved. As now recommended it requires no incubation no humidor cover and may be done in a room regardless of its humidity. For the twelve tests set up at one time a microscopic slide 2 by 3 inches holding twelve paraffin rings is used instead of three slides 1 by 3 inches each holding four paraffin rings. The use of an antigen clear at room temperature and its more thorough immediate mixture with the patient's serum gives better results than the previous method.

CONCLUSION

The microscopic slide precipitation test for syphilis with Kahn's antigen dilution has been further simplified and improved. The immediate test described in this paper, is as specific as the Kahn and Wasserman tests and has the advantage over those methods in that it is much simpler and requires much less apparatus and serum.

REFERENCES

- Kline B. S. and Young A. M. *Jour Am Med Assn* March 27 1926 lxxxv: 928
Green G. *Am Jour Pub Health* 1925 xv: 651

LEUCOCYTIC INDICES OF BODY RESISTANCE WITH REPORT OF A NEW INDEX*

BY WALTER CLINTON JONES, A M, M D, F A C S, AND FRED L CROCKER, B S,
BIRMINGHAM, ALABAMA

THE development of our knowledge concerning the clinical significance of total and differential leucocyte counts, especially in reference to acute infections, has crystallized very largely around five articles by Soderin¹ published in 1905 and 1906. He analyzed a very large series of cases and drew the conclusions that the resistance of the patient is measured by the total number of leucocytes, and the severity of the inflammation, by the polymor

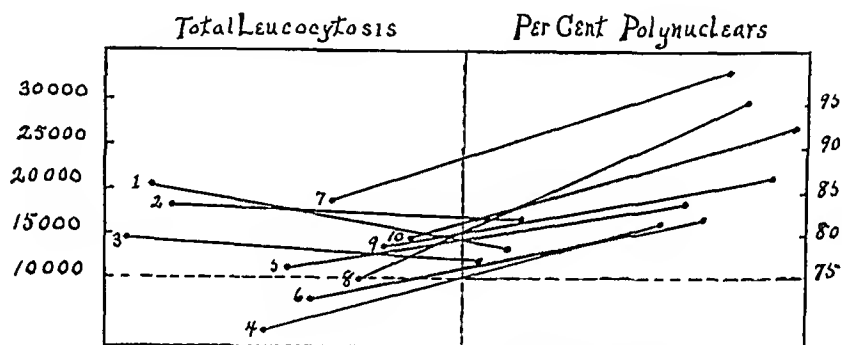


Fig 1—Gibson's chart of leucocytic indices. The left side is used for recording the total counts and the right side the percentage of polymorphonuclears. The numerals in the vertical row at the left indicate the size of the total counts while numerals similarly arranged at the right designate the various percentages of polymorphonuclears. The numerals on the two sides are arranged so that a rise of 1 per cent in the polymorphonuclears corresponds to an increase of 1000 in the total white count starting with 75 as the highest possible normal percentage of polymorphonuclears and 10,000 as the largest possible normal number of leucocytes. The arabic numerals (1 to 10) at the left ends of the index lines indicate the various counts which were made. If the two leucocytic elements are increasing or decreasing proportionately the lines are approximately horizontal and indicate a normal resistance (Counts 2 and 3). If the total count is higher than the percentage of polymorphonuclears the line runs downward and indicates an especially favorable condition (Count 1). If the total count is lower than the polynuclear percentage the line runs upward and signifies an unfavorable prognosis (Counts 4 5 6 7 8 9 and 10).

phonuclear percentage. These probably are the two most fundamental hematologic principles on which depend the clinical interpretation of leucocytic counts.

The first attempt to correlate certain elements of leucocytic enumerations for the purpose of working out a clinical index of the patient's condition was made, as far as we can ascertain, by Gibson,² in 1906. He contended that the chief value of the total count and the percentage of polymorphonuclears consisted in considering them strictly together and constructed an ingenious

*From the Department of Clinical Pathology of the Employees Hospital of the Tennessee Coal Iron & Railroad Company, Fairfield, Alabama.

Read before the Chattahoochee Valley Medical and Surgical Association at the annual session, Warm Springs, Georgia, July 13-14, 1926.

Received for publication July 23, 1926.

chart for this purpose (See Fig 1) When only a small number of counts are recorded, it serves its end without serious difficulties. As the counts increase in number, however, the lines become so numerous and are superimposed upon each other to such an extent that it is difficult to follow consecutively the various counts. Nevertheless, this chart has served a very

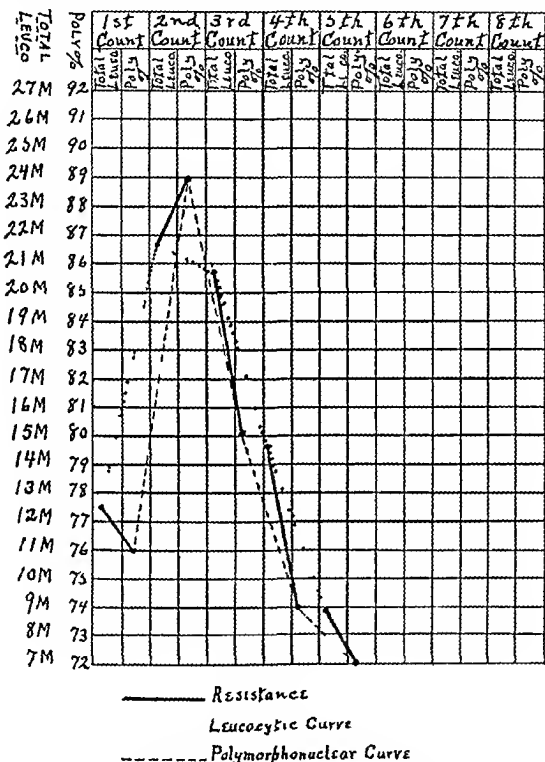


Fig —Wilson's chart of leucocytic indices. When the resistance (heavy) lines slant upward the prognosis is good when downward it is bad. For further description see text.

useful purpose, and to Gibson belongs great credit for originating such an important principle of procedure.

Wilson³ (1908) modified Gibson's chart by making two vertical columns for each count, the left for the total count and the right for the percent age of polymorphonuclears, the same as in Gibson's chart, but Wilson made the columns narrower (See Fig 2). Very few of the main index lines cross each other hence, one can read the tables with perfect ease no matter

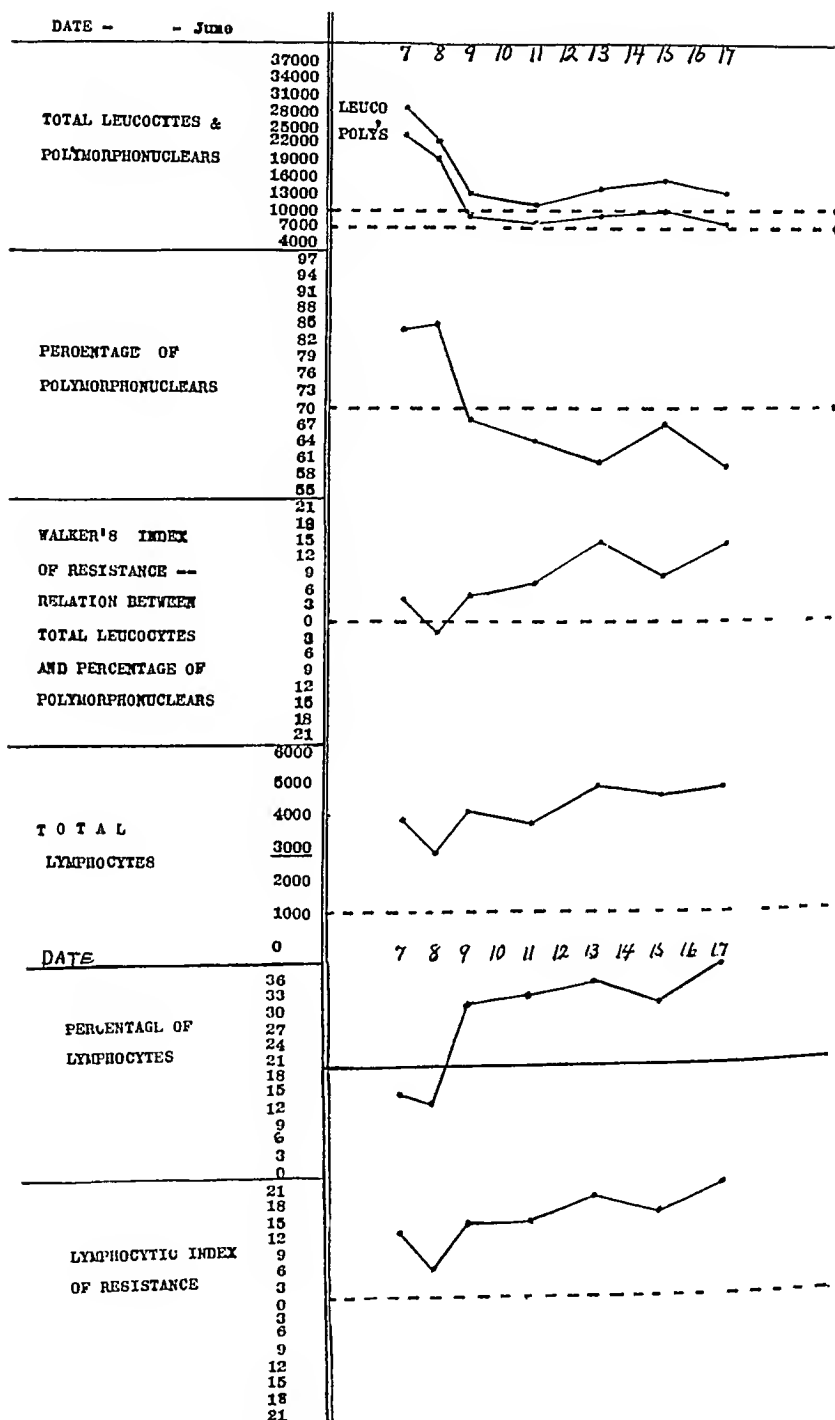


Fig 4—Eclampsia. Age of patient thirty years. Convulsions occurred June 6 and a cesarean section was done the same day. The fall of the indices June 8 might be attributable to the mental and nervous depression caused by the death of the baby. From this point recovery was uneventful save more or less rise in temperature from the thirteenth to the eighteenth day the highest point being 101°. During the first half of this period the indices fell somewhat. For general description of chart see Fig 3. For computation of indices of indices etc., see text.

lymphocytes per cubic millimeter considered alone, independent of any other details of a blood count. While this point is mentioned by many writers, Holmes⁷ (1905) considers it more thoroughly than any one else. He emphasizes the importance of studying the small lymphocytes, although most of the other writers include all of the lymphocytes both large and small while Gilbert⁸ grouped together for the purpose of diagnosis all of the lymphocytes, the large mononuclears, and the transitionals also. Holmes also stresses the value of knowing the number per cubic millimeter rather than the percentage. He alone points out the important fact that the percentage may deviate greatly while at the same time the total number varies within narrow limits. The normal number of small lymphocytes per cubic millimeter he states to be from 1100 to 3000.

In a very large variety of acute and chronic conditions and diseases these various mononuclear elements were found by Holmes and many others to increase almost without exception when the patient was progressing favorably and to decrease when the outlook was bad. In tuberculosis, Gilbert,⁸ Ullom and Craig,⁹ Morgau,¹⁰ and Murphy and Ellis¹¹ all hold that an increase in the lymphocytes (entire group) corresponds with an increase in the resistance of the patient and that a low count almost always is accompanied or followed by poor clinical progress. Hess¹² found that in children a drop in the neutrophil count with an increase in the lymphocytes indicates a good prognosis. Murphy and Sturm¹³ seem to have shown by very conclusive experimental work with mice that one of the constant factors in immunity to cancer is the presence of lymphocytes, especially locally at the site of inoculation. Gilbert⁸ has demonstrated that higher altitudes and Taylor¹⁴ that exposure to sunlight usually produce an increase in the lymphocytes of the blood, while Sauer¹⁵ discovered a lymphocytosis in functional nervous diseases, especially hysteria and neurasthenia.

Of the various indices described above, we favor Wilson's and especially Walker's.

PERSONAL INVESTIGATIONS

1 *The Lymphocytic Index*—In studying numerous charts like those shown in Figs 3 to 6, the senior author noted that a significant curve could be constructed by using only the total white count and the percentage of lymphocytes. (See Figs 3, 4, 5, and 6.) This curve may be designated the 'lymphocytic index' while Walker's might be called the polymorphonuclear index. Our index is computed as follows. After an extensive study of the subject we came to the conclusion that the smallest possible normal percentage of lymphocytes is about 20. Using this figure as the lowest limit for every drop of 1 per cent of the lymphocytes below this point there should be a rise of 1000 in the total count above 10 000 the highest possible normal for the latter. For example if the lymphocytic percentage should fall to 14 (6 below 20), the total leucocytes should rise to 16 000 (6000 above 10 000). In this case the index would be zero, or normal. If the total count goes higher than this, the index is plus, if it falls lower the index is minus. Thus in the above illustration if the total count had risen to 23 000 (the percentage of

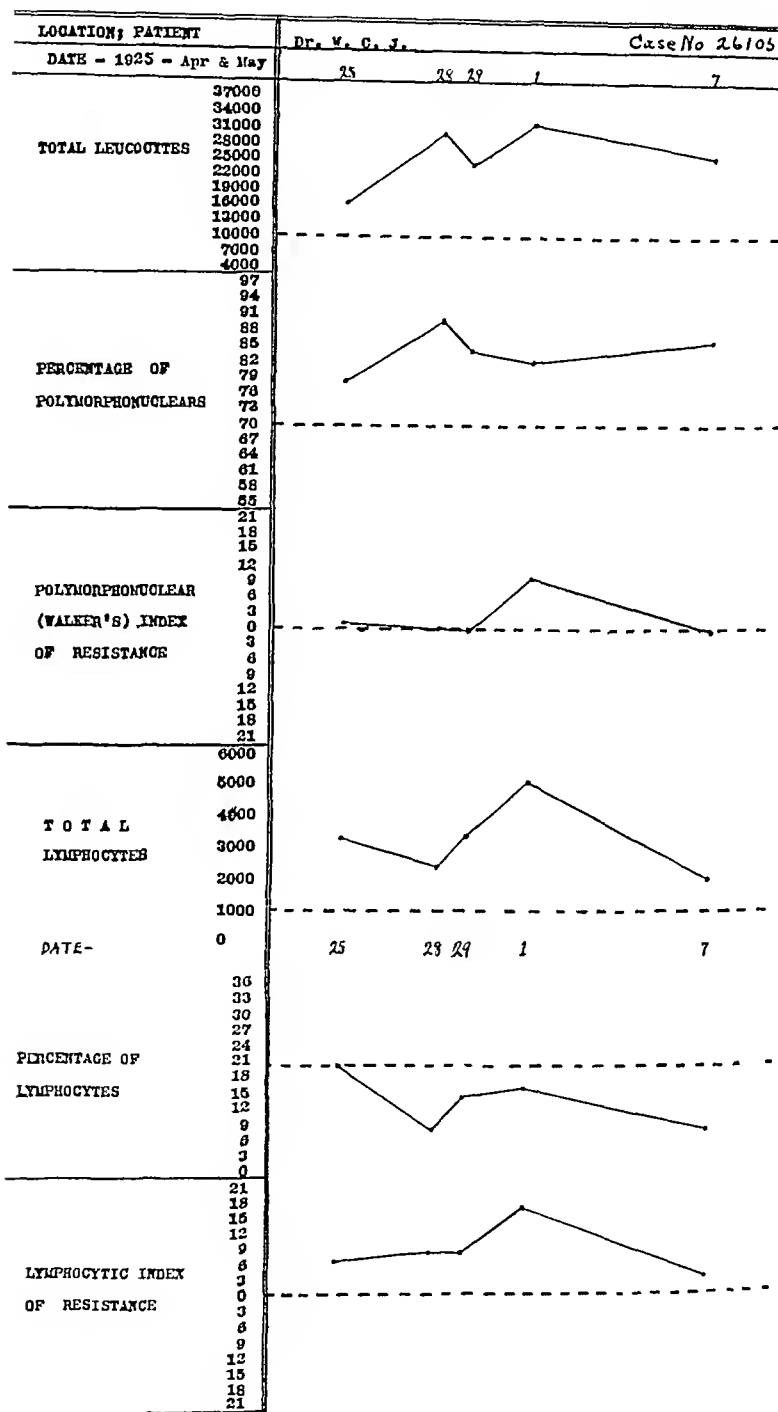


Fig 5—Carbuncle of neck. The patient who was fifty-one years of age grew worse from April 25 to 28 when an excision was done. Note the rise of the indices soon after this operation. They correspond very closely to excellent clinical improvement. From two to six per cent of eosinophiles were present constantly in this case. This is a good omen. For general description of chart, see Fig 3. For computation of indices etc. see text.

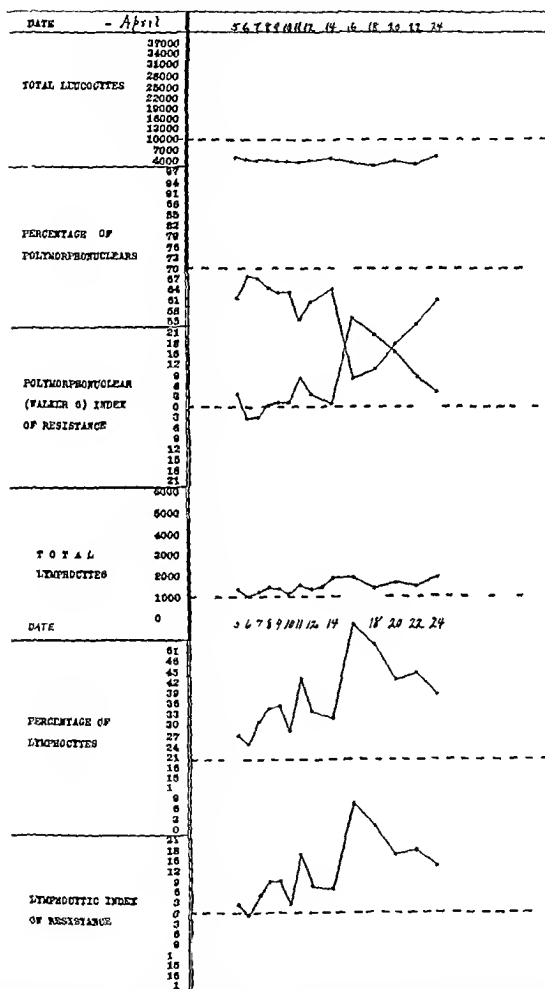


Fig. 6—A case of typhoid fever which ran an ordinary course with recovery. Note that the total leucocytes and the total lymphocytes remain almost constant. This fact explains the almost mechanically accurate interdigitation of polymorphonuclears with the polymorphonuclear and lymphocytic indices and with the percentage of lymphocytes. This chart is a perfectly typical one for typhoid fever. For general description see Fig. 3. For computation of indices, etc., see text.

lymphocytes still remaining at 14), the index would have been +7 (23,000 minus 16,000, omitting the three final ciphers) Similarly, if the total count had failed to go higher than 9000, the index would have been -7

We find that the lymphocytic index averages about six points higher than the polymorphonuclear one Why this is true we have not been able to explain adequately It seemed at first that this difference might be due to the exclusion in our calculations of the endothelial leucocytes, eosinophiles, etc., but on adding together the percentages of polymorphonuclears and lymphocytes and subtracting this sum from 100, the results average about 4, which is only two-thirds of 6, the figure representing the disparity in height of our index above Walker's The two indices closely parallel each other, as one can observe by referring to Figs 3 to 6 Also, they are followed very closely by the curve of the total lymphocytes, as we have shown in a former article⁵

As to the prognostic value of the lymphocytic index, in many instances it seems to be more accurate than Walker's, and in some cases the latter is better On the whole, we believe the best results are obtained by using an average of both

2 *When to Use Leucocytic Indices*—The blood findings discussed in this paper are most significant in acute infections when the percentage of polymorphonuclears is 70 or above and that of the lymphocytes, 20 or lower However, the indices are of definite value in typhoid, tuberculosis, etc., this is especially true of the curve of the total lymphocytes, and the lymphocytic index

3 *Extreme Leucocytic Findings*—Percentages of polymorphonuclears above 90 should be accompanied by total counts high enough to make a plus index of 15 or more, in order to have a favorable prognosis For example, in a recent case of pneumonia, the polymorphonuclears rose to 96 per cent, but the total count was 79,000 These figures give a lymphocytic index of 50 and a polymorphonuclear index of 40 This patient recovered promptly Patients with percentages of polymorphonuclears as high as this usually die, the total white count generally ranging below 50,000

4 *Rules for Normals in Children*—During the first year, the normal percentage of lymphocytes is about 55 and of the polymorphonuclears, about 30 The former figure gradually decreases and the latter increases until about the eighth year when they become almost exactly reversed The senior author has formulated the following rules for ages up to the eighth year For polymorphonuclears After the first year, multiply the number of the year by 3 and add this product to 30 Thus, the percentage of polymorphonuclears at five years of age should be about $(5 \times 3) + 30$, or 45 For lymphocytes After the first year, multiply the year by 3 and subtract this product from 55 For example, the number of lymphocytes at the age of 6 should be about $55 - (6 \times 3)$, or 37 After the eighth year, the percentages are practically the same as in adults

5 *Eosinophiles*—These leucocytes usually disappear during an acute infection. However, their persistence or their reappearance is practically all ways of favorable significance.

6 *Correlation of Blood Findings with Clinical Symptoms*—It is frequently necessary to emphasize this feature. Mere following of blood indices will lead to many errors. For example, both a normal and a seriously sick person may have a zero (normal) index. It is self evident that we cannot have the same attitude toward both of them.

SUMMARY

1 The fact that the percentage of polymorphonuclears is a measure of the severity of an acute infection and that the total white count indicates the degree of the resistance of the patient constitute the two most fundamental principles underlying the application of leucocytic counts to clinical cases. These principles were established about twenty years ago by Sondern, and on the basis of these discoveries Gibson and Wilson worked out very useful clinical index charts.

2 The total number of lymphocytes per cubic millimeter (not percentage) constitutes a valuable clinical index. Holmes has had a very large part in establishing this principle. This index is useful in both acute and chronic infections but particularly in the latter, especially tuberculosis.

3 A lymphocytic index of resistance has been worked out by the senior author, using the total white count and the percentage of lymphocytes. This index parallels Walker's very closely but averages about 6 points higher.

4 Polynuclear indices are useful chiefly in acute infections. Lymphocytic indices are of value in both acute and chronic inflammations.

5 When the percentage of polymorphonuclears rises much above 90, the total white count should reach 50,000 or more in order to justify a favorable prognosis.

6 In children during the first year the normal percentage of polymorphonuclears is about 30 and of the lymphocytes about 55. These figures gradually interchange until they become reversed during the eighth year.

7 The continued presence of eosinophiles or their reappearance during the course of an acute infection is a favorable sign.

8 One should not fail to correlate carefully laboratory blood findings with the clinical symptoms.

REFERENCES

- ¹Sondern, F. E. The Present Attitude of Blood Examinations for Diagnostic Purposes, *Bost. Med. and Surg. Jour.* 1905, cliii 690. The Present Status of Blood Examination in Surgical Diagnosis, *Med. Rec.* 1907, lxxv 45. Value of the Blood Count in Sepsis, *New York Med. Jour.* 1906, lxxviii 121. The Value of the Differential Leucocyte Count in Diagnosis, *Am. Jour. Med. Sc.* 1906, cxxxii 839. The Value of the Differential Leucocyte Count in Gynecology and Abdominal Surgery, *Med. Rec.* 1906, lxx 939.
- ²Gibson, C. L. The Value of the Leucocyte Count in Acute Surgical Conditions, *Ann. Surg.* 1906, xliii 48.
- ³Wilson, L. B. Value of Sondern's Leucocyte Resistance Line in the Diagnosis and Prognosis of Acute Appendicitis, *Collected Papers, Mayo Clinic* 1905-09, Philadelphia W. B. Saunders Co., pp. 280-29.

- ⁴Walker, O J Index of Body Resistance in Acute Inflammatory Processes Indicated by Examination of the Blood, Jour Am Med Assn, 1919, LXXII, 1453
- ⁵Jones, W C, and Brown, C E The Clinical Significance of Total and Differential Leucocyte Counts, with Special Reference to Acute Infections, Am Jour Med Sc, 1922, CLXIV, 553
- ⁶Audain, G Leucocytosis in Infections, Presse med, 1919, XLVII, 1707
- ⁷Holmes, A M The Nature and Significance of Leucocytosis, Jour Am Med Assn, 1905, XLV, 257
- ⁸Gilbert, O M Differential Leucocytes at Various Altitudes, Colorado Med, 1911, VII, 418
- ⁹Ullom, J T, and Craig, F A Examination of Blood in Pulmonary Tuberculosis, Am Jour Med Sc, September, 1905, p 386
- ¹⁰Morgan, E A Study of Blood in Tuberculous Meningitis, Am Jour Dis Child, 1916, VI, 224
- ¹¹Murphy, J B, and Ellis, W M Lymphocytes as a Factor of Resistance, Jour Exper Med, 1914, XX, 397
- ¹²Hess, J H Leucocyte Counts in Pneumonia and Cerebrospinal Meningitis, Am Jour Dis Child, 1914, VIII, 123
- ¹³Murphy, J B, and Sturm, E The Lymphocytes in Natural and Induced Resistance to Transplanted Cancer, Jour Exper Med, 1919, XLIX, 25
- ¹⁴Taylor, H Effect of Exposure to Sun on Lymphocytes, Jour Exper Med, 1919, XLIX, 41
- ¹⁵Sauer, H Ueber das Vorkommen einer Lymphocytose im Blutbild insbesondere bei den funktionell nervösen Leiden und dessen diagnostische und klinische Verwertung, Deutsch Ztschr f Nervenhe, 1913, XLV, 447

THE DIFFICULTIES AND VALUE OF FROZEN SECTION METHODS*

BY L A TURLEY, A M, PH D, NORMAN, OKLA

AT EVERY meeting of this Society in the last several years, the question as to the value of frozen sections in tissue diagnosis has come up and the society has been pretty much divided into two camps, those in favor and those opposed to frozen sections. At the meeting last year in Philadelphia, if you will remember, I promised to present at this meeting a discussion of frozen section methods and it is to keep this promise that I am presenting this paper.

The purpose of any histo-technical method, is to enable us to get a clear conception of the structure of cells and tissues and their relation and arrangement in organs. Therefore, any method must be judged on the basis of the exactness of the picture which it affords. Every one who is familiar with the usual embedding methods or any one who has studied histology and organology from sections made by these methods, has been more or less dissatisfied for a number of reasons. First, it is a long drawn out process, in some cases requiring weeks of time and in many instances requiring infinite care and patience with the individual steps, and second, any of these methods yield results which, beyond any doubt, give a distorted picture of what the tissues actually look like in life. These difficulties arise, because in the first place, the tissues must be subjected to the action of some agent known as a fixative which preserves the tissues in the exact condition they were in when the specimen was taken. These fixatives coagulate or precipitate certain of the protoplasmic constituents and also cause a shrinkage of certain tissue cells, and elements, more notably smooth muscle and collagen fibrils, and if applied in too great concentration will cause the shrinkage of

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas Texas April 15 16 and 17 1926

cells, for example, certain types of epithelium, hence the distortion is apt to be very great. Second, since all embedding substances are insoluble in water, it is necessary to dehydrate the tissues before they can be embedded and cut which again gives rise to possibility of distortion unless great care is exercised to not hasten the dehydration process. Third, after tissues are cut it is necessary to rehydrate them in order to stain them and again dehydrate them for mounting so that it is perfectly evident that this method must be carried out with utmost care in order to get sections that are at all satisfactory and even then we must recognize that the tissues are somewhat distorted. These disadvantages are offset to a certain extent by the accurate preservation of tissue structure and even of intracellular structure both of which are of extreme importance in obtaining an accurate knowledge of tissue structure. In fact so important are these structures to an exact knowledge of tissues that their preservation outweighs practically all objections to the embedding method although it is necessary at all times to remember that we are looking at distorted specimens. Another point in favor of this method is the retention of the chromatophilia of the cells and cell elements which are highly differentiated without a view of which the fine points of tissue structure and cellular identification cannot be determined. But it is quite obvious that such a method whatever its merits because of the time involved, never could be used if a rapid diagnosis of tissue were necessary.

With the discovery and development of the frozen section method histologists and histology technicians at once expected emancipation from the difficulties attendant upon the embedding method. However they are doomed to disappointment for many reasons. In the first place in the process of freezing and thawing there is bound to be more or less loss of tissue continuity and even the loss of a great many if not of all parenchymatous cells in sections of organs and from lesions where the stroma is scanty and second in the handling of these unsupported delicate sections tissue relations are often distorted for example too strong a pull with a camel's hair brush or a glass rod on one side of a section will make the tissues appear edematous. It was also found that unfixed tissues do not stain as readily nor as sharply as do fixed tissues so that fine points of intracellular and intercellular structure cannot be made out. However there is an increasing demand on the part of clinicians for an exact knowledge of the nature of lesions with which they are dealing especially in surgical procedure. At the Mayo Clinic surgical specimens are examined and the surgeon furnished with a diagnosis while the patient is under ether. Dr. Bloodgood has the staining and examination process performed in the operating room so that he not only is furnished with the diagnosis during the operation but is enabled if he so desires to step to the microscope and see the character of the lesion himself.

A careful and conscientious surgeon wants to and all surgeons should as nearly as possible know the nature of the lesion with which he is dealing while the patient is under the influence of ether and the wound open so that he will know how to proceed. An examination of the records of any hospital clinic or private practice will show that mistakes in diagnosis are made

without tissue examination and procedures carried out on the basis of those diagnoses that not only fail to benefit the patient, but often injure him, sometimes hastening his demise. I need not cite these examples from my own experience. Look at the records of your own clinics and you will find them. These mistakes are often made when every known diagnostic method except tissue examination is employed. This is no criticism of surgeons, clinicians, nor clinical pathologists. These mistakes are due to the finiteness of human knowledge and the lack of employing the right diagnostic aid. If it were not for the first of these factors we would not be here, for clinical pathology would not be a branch of medicine. No surgeon can tell the exact nature of a lesion in every case without tissue examination any more than an internist can tell in every case the lesions with which he is dealing without the aid of laboratory procedure. The tissue examination is to the surgeon what the Wassermann, Widal, urinalysis, blood chemistry, basal metabolism, etc., is to the internist. Therefore, it is imperative that some method of rapid tissue diagnosis be devised that is much shorter than the embedding method, and it falls upon us as clinical pathologists, in the fulfillment of our calling as the makers and interpreters of exact diagnosis and in justice to the patient and clinician to devise and employ some method of rapid tissue diagnosis that is suitable for diagnoses while the patient is under the ether. For this reason a discussion of methods of rapid tissue diagnosis is not only timely and desirable, but becomes as much our duty, and should be as much our interest, as the discussion of any laboratory method, bacteriologic, serologic or chemical. It does not get us anywhere to dwell on the difficulties of rapid tissue diagnosis, and recount the failures or possible inaccuracies of the method unless we at the same time attempt to devise means for overcoming those difficulties and minimizing the number of possible inaccuracies. What clinical pathologic method is 100 per cent perfect? Over one-half of one of our programs was taken up with discussions of the Wassermann reaction and suggestions as to how it could be made more exact and reliable and yet it is perhaps the most reliable of all laboratory procedures. In the few minutes of time that remains to me, therefore, I wish to bring out some of the difficulties of frozen section methods and suggest ways in which these difficulties can best be combated or minimized and also the methods for obtaining the best results.

In the first place, in freezing blocks of tissue, care must be taken not to freeze the tissues too hard or cut them while they are frozen too hard because during the thawing out process the rapid change in temperature will set up currents which will tear to pieces tissue composed of unsupported cells. I am sure that all who have tried the frozen method have seen sections, when placed in water from the knife, fly to pieces almost as if they had exploded. Therefore the temperature range must not be too great and the thawing process should not proceed too rapidly. This can be brought about by not freezing the tissues harder than is necessary for actual cutting and the slow thawing can be accomplished by either allowing the section to thaw on the knife, preferably, in a drop of liquid that can be carried on the

knife, or as Terry recommends, on the finger and if this detail is observed one will be surprised how perfect a section, even of very cellular tissue, may be obtained by the frozen method. In the second place, further tissue distortion can be obviated by care in handling. If the sections are to be mounted on slides by the use of egg albumen or otherwise they should be floated and not dragged into position. It is remarkable what a perfect tissue arrangement can be preserved by this procedure.

We now come to the staining. It is perfectly obvious that the staining and clearing process must be done quickly otherwise much time is lost. But it is just as obvious that any staining method used must give a cellular differentiation clear enough that the tissues may be quickly recognized and their condition determined and further it is necessary that the preparation be of a permanent character so that it may be mounted and preserved for as has been pointed out before the slide from which a diagnosis is made is as much a part of the record in a case as the diagnosis itself. For general all round purposes, no method of staining has yet been devised that surpasses or that is equal to the hematoxylin eosin method. But this is rather an elaborate method requiring a good many steps so that the actual staining by this method requires ordinarily two or three minutes as a minimum which of course seems a long time to a surgeon standing beside a patient under ether and waiting to know how to proceed. Various attempts at developing shorter methods by use of other methods and especially using polychrome stains have been tried and although some of them are very rapid they lose so much of detailed differentiation that a diagnosis from such a stained specimen is much more difficult to make and time gained in the staining is lost in the additional time required in the examination of the stained specimen. Terry reports that by his polychrome method a section may be stained in ten seconds and McCarty using this method has stated that he has made a diagnosis in fifty two seconds from the knife but in Terry's own words it is practically necessary for the pathologist to learn a new cytologic interpretation because of the lack of sharp differentiation and also that these sections fade in a few hours and must be restained by another method if a permanent record is desired, which as pointed out above is all important which means additional work that is unnecessary if speed and permanence can be obtained by the one process.

Realizing the necessities the criticisms and aims of frozen section work the author conducted a series of experiments trying if possible to obtain a section under the microscope with a good differentiation in a very short time. Of course the interpretation of a slide picture depends upon the skill of the pathologist. The time the surgeon has to wait for a diagnosis depends not on the staining method alone but also on the skill of the pathologist in interpreting the slide picture. The staining method is employed merely as an aid to the pathologist. Therefore it should be as rapid as possible to give him a larger percentage of a given time to examine the specimen. Also since it is merely an aid to give him a picture it must be as clear as possible in fact clearness of the picture is the more important of the two considerations.

And in the end more time will be saved if a clearer picture is obtained by a little longer process so that the examination may be shortened, than by a very rapid process which gives a hazy picture which requires more time to study. So the aim of rapid procedures should be the shortest possible process that gives clear pictures. This was the aim of the author in the following procedures.

Starting with the fact that fixed tissues give good clear pictures by the frozen method, an attempt was made to introduce a fixing process into the frozen method. It was found that if sections, after being mounted on a slide and before the staining was begun, were flooded with 4 per cent formaldehyde for a few seconds, the subsequent stain was much faster, clearer and more definite. Therefore experiments were tried floating the sections from the knife in formaldehyde solutions of various strengths. It was found that formaldehyde in concentration of 4 per cent could be used in place of water and this accomplished several things. First, a fixation which preserved tissue structures in as near as possible the natural condition in which they were in the body, second, by the coagulation of intercellular and intracellular structures the tissues do not tend to disintegrate after being cut, and resist the deleterious effects of handling to a very great degree, and third, increased the speed and sharpness of the stain. Emboldened by the rigidity and toughness of the sections thus treated, experiments were carried on to see if the mounting process could not be delayed until after the staining and clearing, thereby improving the stain both as to shortness of time and greater exactness because the time necessary to fix the section to the slide would be saved and the cells would stain from both sides of the section instead of from one, as is in the case of the mounted section. To our great satisfaction it was found that this could be done, so here again considerable time is saved and mounting media could be dispensed with. The method is as follows. Sections are thawed on the knife by cutting them into the small amount of fluid that will cling to the upper side of the knife previously immersed in the floating fluid. The under side of the knife must be wiped dry. Sections are floated in 4 per cent formaldehyde. A glass rod is employed for handling the sections. This glass rod is drawn into a narrow tip bent at a slight angle. The section is lifted into the hematoxylin solution. In from one to three seconds it is lifted into tap water and dipped up and down a couple of times to wash off the hematoxylin. It is then dipped into 70 per cent acidulated alcohol for differentiation, back into tap water, next ammonia water, tap water, then eosin for two or three seconds, and into 95 per cent alcohol for a second, then into beechwood creosote. If the section is plunged perpendicularly into the creosote it will unfold like the opening of a book and will straighten out, it is then slid onto a slide and can be examined in the creosote with or without a cover glass. If it is desired to preserve the section, the creosote is blotted off and balsam and cover glass are put on. This method gives a clear sharp stain and a permanent mount as permanent as any hematoxylin eosin stained specimen by the long embedding process. I realize that the first criticism of this method that will be made, is that there are an enormous number of steps in the procedure, but

you will be surprised at the short time required by each step, and so far as the time the whole process requires, in the hands of Mr. McHale, an untrained student, I was able to have good clean sections, under the microscope in thirty five seconds, which considering the excellence of the product and its permanence, is a short enough time for anyone. Also the excellence of the product justifies the few seconds more time over the more speedy polychrome methods now in use.

Having a staining method that is accurate and rapid enough for practical purposes there are some other important factors that must be considered if rapid tissue diagnosis is to be as serviceable as it should be. One of these is the selection of the block of tissue to be sectioned. This is in most cases up to the surgeon whether the tissue is sent to the laboratory before the operation or during the operation. Most imperfect diagnoses that are charged to frozen or rapid tissue diagnosis are due to the failure of the one taking the specimen to get a characteristic block of tissue. All that can be expected of the tissue pathologist is that he make an exact diagnosis and interpretation of the specimen furnished him. So if the tissue diagnosis proves to be wrong the first question to raise is not the pathologist's honesty or ability, but the selection of the tissue furnished him. I have on several occasions asked the surgeon for a second piece of tissue even when the patient was under ether and have always justified this delay by a more accurate diagnosis.

In making a tissue report from a small piece furnished for rapid diagnosis, one should always say "Sections from *this specimen* show such and such conditions." Such a statement is the truth; it protects the pathologist from criticism and is more satisfactory to everyone concerned because it is all the pathologist can say and it at once notifies the surgeon that if the diagnosis was not what he expected he has the means of getting more information which is, furnishing another block of tissue from another part of the lesion.

Another important factor to be considered is the pathologist himself. Much of the criticism of frozen section diagnosis from the clinicians is because those without proper training are attempting tissue diagnosis. And much of the reluctance of clinical pathologists to do rapid tissue diagnosis is because they realize their inability to diagnose tissue changes. The recognition of tissues in abnormal conditions and to properly interpret such conditions is the most difficult of all clinical pathologic procedures. It is easy to maintain a constant temperature in a bath to add so many cubic centimeters of this or that solution to another and watch for a precipitation or change of color or reduction of some chemical substance. It is easy to count blood cells to recognize bacteria etc. but to tell when hyperplasia becomes neoplasia to recognize atypical pancreatic cells in the lungs, or atypical mammary cells in the brain is quite a different story. A tissue pathologist must be a cytologist. He must be familiar with the normal appearance of cells in the different phases of their functional life and the history of their development from the undifferentiated cells of the germ layers. He must be familiar with the differences in appearance of cells in inflammations, degen-

erations, regenerations and neoplasias. No one should attempt tissue diagnosis who does not have a thorough knowledge of histology and embryology, followed by long, extensive and careful study of histopathology.

SUMMARY

1 Frozen sections or equally rapid tissue diagnosis is as important as any clinical laboratory procedure, and it is as much up to clinical pathologists to prepare themselves for this service as for any other clinical laboratory procedure if they are entitled to the full respect and place in medicine they should have and take.

2 Good, clear, permanent mounts with good differentiation, sufficient for all clinical diagnoses, can be made in one minute or less, which is short enough time for all practical purposes.

3 We should insist on specimens from such parts of a lesion as will reveal its true character, and in all cases to state that the specimen furnished revealed the facts which led to the diagnosis made, and that the diagnosis is a diagnosis of that specimen only.

4 Tissue diagnosis is the most difficult of all laboratory procedures so should be attempted only by those specially trained.

DISCUSSION

Dr Michael G Wohl—Tissue diagnosis by the frozen method is one of the links that still binds the clinician to the clinical pathologist. It is a branch of clinical pathology the execution of which cannot be delegated to the lab technician or the Board of Health. Tissue interpretation requires a medical mind and clinical judgment that can only be acquired through years of experience. We should therefore endeavor in every case to discuss the clinical side with the surgeon. The clinical pathologist should be just as much interested in the clinical symptoms of the patient as in the laboratory findings. I feel that it is a good rule to follow to be present in the operating room and see the tissue the surgeon examines. When the tissue is cut into one often may be able to tell from the naked eye appearance as to whether or not the tissue is malignant. The clinical pathologist is able to point out to the surgeon the appearance of malignant tissue, and the surgeon will soon become familiar with the appearance of pathologic tissue when he sees it. You thus make a friend of the surgeon. The secret of obtaining good frozen sections is to do them routinely, often enough. We have obtained sections that were thin enough for microphotographs. It seems to me the difficulty lies, not in the preparation of the tissue so much as in the time allowed for the study of a frozen section. When the surgeon desires an immediate diagnosis, I would rather trust an opinion based upon a careful clinical history and the gross appearance of the tissue than a hasty examination of a frozen section.

Dr F W Hartman—It is a good thing for us to consider the whole question of frozen section diagnosis and to get the various ideas regarding the Terry stain. In our own laboratories our results have been variable. At one time a nuclear stain is obtained and at another time no differentiation is brought out even when using the same batch of stain. It has seemed that with the older method of polychroming more consistent results were obtained. Dr Turley's method for quick sections is interesting and well worth a trial. However, it has been our experience that creosote often distorts fresh tissue and the section is often wrinkled. If so much time has to be consumed we would prefer the fixed frozen section stained with hematoxylin and eosin. We cannot agree with Dr Wohl's remarks regarding the pathologist making a diagnosis from the gross tissue alone. If the surgeon is worthy of the name he can make his own gross diagnosis and he wants confirmation of this diagnosis from the pathologist's examination of sections. It should never be necessary to review the history after the patient is on the operating table. This should be done before the operation is started so that the pathologist will be in position to make

the histologic examination at once. In our institution we make it a rule that the frozen section diagnosis shall be tentative only and that a change may be made in this diagnosis if the permanent sections warrant it.

Dr Philip Hilkowitz.—This question of frozen sections is of great importance to the surgeon. There is no field wherein the clinical pathologist is of greater value to the clinician. There is where the surgeon feels a dependence on the clinical pathologist, the life of the patient and the extent of the operation depend on his dictum. I am very glad that Dr Turley has brought this matter up. It is not so much a question of the particular technique employed as it is whether the tissue is benign or malignant. I was rather shocked by Dr Wohl's remarks. For instance in a case of suspected malignancy of the breast where the clinician calls on the clinical pathologist for a diagnosis it is of great importance to the surgeon to know what procedure to follow. If it is a case of adenofibroma it would be a crime if the breast were removed, the tumor could be shellied out and the breast remain. On the other hand if it is carcinoma a radical operation with excision of the axillary lymph nodes would be done. At the same time I fully agree that in the great majority of cases the clinical pathologist is in a position to make a diagnosis with the naked eye. While the surgeon should be able to do it we know from experience he has not skilled himself to that extent. Very frequently for a skilled pathologist it is simply going through the motion to have a frozen section made when he can tell by the naked eye but as a rule it has a good effect to demonstrate the microscopic section to the surgeon and confirm the macroscopic diagnosis.

Dr Hermon Spit.—It seems to me that we are missing the most important point in regard to frozen sections. You will recall that during the first year of our existence as a Society, our president, Dr Hilkowitz carried on quite a protracted discussion with the American Medical Association in regard to advertising. As a result of that discussion, the Advertising Committee of the American Medical Association classified us as "manipulators of test tubes and other inanimate objects." Now it seems to me that here is a splendid opportunity to refute that classification. We are doctors as much so as any other members of the American Medical Association. We are called into consultation by the surgeon when we are asked to do a frozen section. The surgeon waits with bated breath until we give him our report. If we tell him to cut wide he does so. If we tell him he has done enough and that no further surgery is required he is satisfied. Now if this is dealing with "test tubes and inanimate substances" somebody should rewrite the dictionary. In the case of a young girl with a mass in the breast it is up to the pathologist doing the frozen section to say whether that girl should keep her breast or lose it and be disfigured for life. The same argument is true in many other instances. The method of doing a frozen section, the technique employed, the stains used, etc. are matters of personal preference. The real value in this paper is the fact that it tells the Advertising Committee of the American Medical Association that we are real doctors dealing with life, just as all other physicians deal with life and that we do something besides "manipulate test tubes and other inanimate substances."

Dr E. F. Cooke.—I don't believe it makes so much difference which technique is used. I have seen beautiful sections by various techniques and even by freehand cutting. What I object to, is the hurried diagnosis. It is not the obvious case that is important. In such a case the surgeon himself is in no doubt and does not need our aid to make his decision, but it is the borderline case, the one that we even find difficulty in diagnosing after we have our sections cut. I feel sure that all here who do tissue work have right now specimens lying on the table that we are hesitating to express an opinion upon and they may have been lying on the table for a week or ten days. It has been argued that one can make a hurried frozen section diagnosis and then confirm or modify the same after a more deliberate technique and study, but it is quite true of human nature that we hate to take up a task that we thought completed. It is quite a bore to take up a tissue again and go on further with it. And it is quite embarrassing to have to change or modify a diagnosis. About seventeen years ago I could make a prompt diagnosis of any tissue. I cannot do that now, maybe it is because I am getting old and cannot see. Some time ago I had the pleasure of seeing the tissues from the Boas Sarcoma Library of

Atlanta, Ga and it was interesting to see the different diagnoses that had been rendered by different pathologists on the same tissues. This was not only interesting but consoling to a man like myself. I object to the surgeon who wants to hurry you to an express train diagnosis, when he has probably been studying the case for weeks before he dares operate.

Dr L. A. Turley (closing)—Dr Wohl makes two very important points, one of which is that tissue examinations cannot be delegated to lay technicians or Boards of Health. As was pointed out in my paper, tissue diagnosis requires the most careful expert preparation of any branch of clinical laboratory procedure for reasons above pointed out. The other one with regard to Boards of Health, tissue should never be sent to Boards of Health for the reason that they can never be Public Health matters and further no Board of Health staff should or can afford one capable of doing tissue diagnoses. Another point made by Dr Wohl is that frozen sections should be made often enough, whether requested or not by the surgeon, to train the technician in the cutting and staining, and the pathologist himself in interpreting frozen sections.

On some other points of Dr Wohl's discussion, I must heartily disagree. That is, the making of diagnoses from the appearances of gross specimens without microscopic examination. If time permitted, I could cite cases from the best laboratories in the country where the opinion was arrived at by the most expert clinicians and pathologists that the gross diagnosis was proved erroneous by tissue examinations. I am not pessimistic when I say that I doubt whether any pathologist, regardless of his innate ability, his training and experience, however prolonged, is capable of making gross diagnoses that are exact and reliable in a sufficient number of cases to be absolved from a necessity of checking up such diagnoses with tissue examinations. Such a statement is based on the point made above which is the finiteness of human knowledge which will prevent a full appreciation and realization of the varied appearances and conditions met with. Even if one sees the tissue *in situ*, the distortion due to light, mechanical manipulation, presence of hemostats and other instruments, discoloration by hemorrhage, and other factors confuse the picture so that it is more difficult to make a gross diagnosis *in situ* than after the tissues are removed. Even under these circumstances, gross diagnoses cannot be made that are sufficiently reliable to dispense with microscopic examinations. It often happens that malignancies are found on microscopic examination that are not suspected on clinical examination nor gross inspection. Further, as Dr Cooke stated, it is the borderline case and the unsuspected case where the tissue pathologist serves his most useful purpose. If the case is perfectly obvious, the services of the tissue pathologist are not necessary. There are many mounds in cemeteries that are monuments to the conceit of clinician's and pathologist's ability to make diagnoses from clinical facts and gross inspections.

The point brought out by Dr Hartman in his discussion that creosote often wrinkles tissues is true but is no criticism against the frozen section methods. If you will take a binocular microscope and examine sections made by any technique however prolonged and careful, you will find wrinkles that were not suspected and which often distort the picture. However, I think Dr Hartman will find that if sections are fixed as outlined in the paper and a little more time spent in the dehydration process he will have less trouble with the creosote wrinkling.

The point brought out by Dr Cooke with regard to the time often necessary to make a diagnosis is also no criticism of frozen section methods. I have personally known some of the ablest tissue pathologists now living to spend from three weeks to six months studying specimens from a case before they are willing to make a diagnosis even when they had employed not only the long embedding method but also differential stains. The frozen section method is intended to give the greatest amount of information in the shortest space of time and, as stated above, has never and probably never will supplant the longer embedding methods for exact diagnoses. When this is fully understood, I think no one should feel abashed or ashamed of having to revise his diagnosis any more than a clinician should in revising his diagnoses on the basis of more complete and more exact information.

In closing, I will say that the method presented is neither the only one nor perhaps the best one. All that is claimed is that by employing this method one can get a very exact picture and a permanent mount, both of which are of supreme importance in tissue diagnosis.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D. ABSTRACT EDITOR

Mellon R. E. The Infective and Taxonomic Significance of a Newly Described Ascospore Stage for the Fungi of Blastomycosis. Jour. Bacteriol. April, 1926, xi, No. 4 p. 229

In a paper illustrated by 27 microphotographs Mellon describes observations furnishing evidence for the formation of four celled asci with Types I and II of the parasites of blastomycosis as described by Ricketts this perfect stago representing a form of endosporulation not previously described.

This phase of development seems to appear exclusively in the so-called secondary colonies although all varieties of colonies do not contain them. The probable occurrence of asci and related special growth forms in the tissues of the host offers a probable explanation for the recrudescence of the disease after apparent cure.

Demonstration of ascus formation suggests the allocation of these organisms among the ascomycetes rather than with the Oidia or Cryptococci.

Koser S. A. and Galt R. H. The Oxalic Acid Test for Indol. Jour. Bacteriol. April 1926 xi, No. 4 p. 293

The authors extol the advantages of the oxalic acid test for indol first described by Pittaluga in 1908.

Absorbent paper or filter paper is dipped in an aqueous solution of oxalic acid dried and cut into strips and a strip of the paper suspended from the cotton stopper in the mouth of the tube containing the culture to be tested.

As large a surface as possible should be exposed but the paper must not come into contact with the culture.

If indol is formed it volatilizes at either room or incubator temperature and the oxalic acid paper becomes pink.

In the absence of indol the paper remains white.

The test has several advantages:

- 1 Nonvolatile compounds related to indol are eliminated.
- 2 The culture is not destroyed.
- 3 Repeated tests can be made.
- 4 Tests can be applied to cultures on solid media.

The test has not given false positive reactions and is fairly delicate though not so delicate as the Gore test.

Magoon O. A. Studies upon Bacterial Spores. I Thermal Resistance as Affected by Age and Environment. Jour. Bacteriol. April 1926, xi, No. 4, p. 253

Studies were conducted upon spore suspensions of *Bacillus mycoides*.

The following method was developed for the preparation of the spore suspensions.

Clean fine quartz sand such as is used in greenhouse experiments, was passed through a standard 40 mesh brass sieve subjected to thorough washing and dried. Twenty five gram quantities were then measured into 15 x 100 mm. Petri dishes distributed over the bottom of the plate in an even depth and sterilized in the dry air hot oven.

The sand serves as the substratum for the culture.

A suspension of spores from an old agar culture is made in standard beef extract peptone broth (Manual of Methods for Pure Culture of Bacteria 1923) and boiled. With a sterile pipette and aseptic precautions just enough of this suspension was transferred to the sand plates to exactly saturate the sand and the cultures thus prepared incubated at 37°C.

The spores were thus suspended in a highly favorable medium and maximum germination was assured.

Hourly observations were made to determine the length of the spore cycle.

For the thermal resistance tests standard suspensions were prepared as follows:

About 20 cc of sterile distilled water was transferred aseptically to a sterile 20 x 150 mm culture tube. With a freshly flamed small, flat edged metal scoop a quantity was introduced into the tube which is then plugged, and agitated until a sufficient number of spores have been freed into the liquid. The suspension is then transferred with sterile precautions to a sterile centrifuge tube and centrifuged at high speed. The sediment is resuspended in sterile water and again centrifuged, this washing being twice repeated. The washed spores are finally suspended in sterile distilled water and standardized by opacity.

The standardized suspension is then transferred with aseptic precautions to a small sterile shell vial inserted into modeling clay at a convenient angle.

Pyrex tubing with an internal diameter of 4 mm was drawn into capillary tubes about 9 to 10 cm long with an internal diameter of 1 to 1.5 mm, and sealed.

A sufficient number of such tubes were placed in a glass Stender dish and covered with alcohol. As needed, a tube was removed with flamed forceps, the alcohol burned off, and the sealed ends clipped off by a sterile instrument. The tube is then dipped into the spore suspension, filled by capillary attraction, and resealed, the fluid being first centered to leave an air space at each end. The sealed and filled tubes were then dropped into cold potassium bichromate solution to sterilize the outside.

The solution is then washed off in cold water and the tubes placed in fresh alcohol chilled by placing the dish on crushed ice. Any tendency toward germination with resultant loss of heat resistance is thus obviated.

Heat resistance was tested by exposure in an oil bath, each test being made with five such tubes which, after removal from the bath, were placed in acetone in a 4 ounce salt mouth bottle to remove the oil. They are then placed in alcohol until inoculated.

The sterility tests were made by flaming the plug and mouth of the culture tube in the usual way and then by introducing the capillary tube containing the spore suspension. In preparing the capillary tubes for this inoculation they were withdrawn from the alcohol with sterile forceps and, without flaming, one sealed tip was removed with the freshly flamed clipper. The open end was then inverted over the mouth of the culture tube from which the cotton plug has been removed, and the upper tip of the capillary snipped off. At the same instant the capillary tube was released and dropped into the nutrient broth of the culture tube. The plug was then replaced, and after making certain that the contents of the capillary had been forced up into the medium by the bubble of air formed when the tube touched the medium, the culture was ready for incubation.

A careful analysis of the experimental data presented leads to the following conclusions:

- 1 The bacterial spore is not dormant under ordinary conditions, as has commonly been supposed, but is, instead, sluggishly active.

- 2 The resistance of spores to heat is not a fixed property but a variable one, the degree of resistance being influenced by age, the temperature and humidity of the environment, and possibly by other factors.

- 3 The highest resistance to heat develops under conditions of moderate temperature and humidity, and is probably reached by the time the spores are sixty days old. Spores of different species of bacteria may be expected to vary somewhat in this respect.

- 4 Change in resistance takes place most slowly when spores are dry and cold, but low temperature accompanied by high humidity results in the development of a high degree of resistance.

- 5 In determining the thermal death points of spores that are to serve as the basis of processing schedules for canned foods the bacteriologist must take into account the change in resistance of spores under various conditions, and be as certain as possible that the resistance shown by the test spores represents the highest degree attainable by them.

The paper includes an extensive bibliography.

St John J H Practical Value of Examination for *Endameba Histolytica* by Culture. Jour Am Med Assn, April 2, 1926, LXXXI 1272

Medium—Four whole eggs are broken into a sterile receptacle Fifty cubic centimeters of Locke's solution (NaCl 9 gm, CaCl₂ 0.24 gm, KCl, 0.42 gm, sodium bicarbonate, 0.2 gm, glucose, 1 gm, distilled water 1,000 cc), are added and the whole emulsified

Place about 10 cc of the emulsion in sterile tubes, incline and heat to about 70 °C until solidified Sterilize in the autoclave for twenty minutes at fifteen pounds pressure To the solid medium now add sterile inactivated human or horse serum diluted seven times in volume with sterile Locke's solution The liquid layer should cover the whole or part of the slant

Inoculate by rubbing against the wall of the tube a selected sample the size of a pea

The medium must have been inoculated to insure sterility and should be warmed in the incubator before use

Incubate at 37 °C and examine by taking a drop of fluid from the bottom of the tube

By this method ameba have been grown from specimens transmitted by mail (forty eight hours) and after eight days in the ice box

Glenny A T Pope C G Waddington H and Wallace N The Antigenic Value of the Toxin Antitoxin Precipitate of *Ramon* Jour Path and Bacteriol January 1926 xxix, 31

A report of studies upon the antigenic value of the precipitate which occurs when diphtheria toxin and antitoxin are mixed, the following observations being recorded

1 An emulsion of the toxin antitoxin precipitate was equally efficient in doses of 0.001, 0.1, or 1 cc, thus suggesting that the antigenic value of the precipitate depends upon the rate of dissociation of the toxin antitoxin complex after injection

2 The destructive action of heat is greater on toxin containing 0.5 per cent phenol than on uncarbolized toxin The presence of trikresol also increases the rate of destruction by heat

3 Neutral mixtures of toxin and antitoxin may become toxic upon evaporation because the increased concentration of phenol destroys antitoxin at a greater rate than toxin

4 The addition of 0.2 per cent formaldehyde destroys half the antitoxic value of a serum

5 The antigenic value of toxoid is slightly increased when precipitated by the addition of 1 per cent glacial acetic acid Toxin or toxoid may be precipitated by the addition of varying quantities of potassium alum An emulsion of such a precipitate has a high antigenic value

6 Some batches of toxoid of high antigenic efficiency may fail to flocculate but the absence of flocculation does not necessarily indicate the absence of combining power

Julianelle L A and Reimann H A The Production of Purpura by Derivatives of *Pneumococcus* I General Considerations of the Reaction Jour Exper Med January, 1926 xliii 87

A study of the nature of the hemorrhagic purpura produced in white mice by the injection of pneumococcus extract

If pneumococcus extracts are injected into white mice, within four to six hours the skin over the feet, tails ears snout, and genitals take on a dark bluish purple color most marked where the hair is naturally scanty or absent

There are no signs of intoxication, the reaction reaching its maximum in twenty four to forty eight hours and vanishing in five to seven days

The reaction follows any method of injection but does not follow feeding the extract

The purpura producing principle resists heating to 100 °C for ten minutes, resists oxidation is filter passing and is destroyed by trypsin

It is obtained from pneumococcus extracts by full saturation with ammonium sulphate after the acetic acid precipitable substances are removed

It is common to all types of pneumococci and bears no apparent relation to virulence and is probably a degradation product of pneumococcus and is not associated with pneumococcus hemotoxin

Reimann and Julianelle (II The Effect of Pneumococcus Extract on the Blood Platelets and Corpuseles Jour Exper Med, January, 1926, *Vol.* 87) found that the injection of the extract caused a marked reduction in the number of blood platelets and that purpuric lesions usually developed when the blood platelets dropped below 500,000 per c mm

The red cells were also reduced in number, but their destruction and regeneration were somewhat slower The leucocytes were affected only slightly, if at all

The extracts were both thrombolytic and hemolytic Heated extract produced purpura but not anemia Extracts adsorbed with blood or platelets showed a decreased thrombotic and hemolytic activity but were still able to produce purpura as well as severe anemia and thrombopenia

Gordon, J, and M'Lead, J W Inhibition of Bacterial Growth by Some Amino Acids and Its Bearing on the Use of Tryptic Digests as Culture Media Jour Path and Bacteriol, January, 1926, *Vol.* 13

These studies are concerned with the effect of the addition of amino acids to ordinary peptone broth, upon the growth of bacteria difficult to cultivate

Fourteen different amino acids were studied, as a result of which it was concluded that, in the absence of serum, etc, medium with a basis of tryptic digest are inferior to peptone broth for growing delicate bacteria, but that such media can be improved if a considerable part of the amino acid is removed by butyl alcohol extraction

Perlzweig, W A, and Keefer, C S The Immunization of the Pneumococcus III The Purification of the Water-Soluble Antigen Jour Exper Med, December, 1925, *Vol.* 41, No 6, p 747

Actively immunizing fractions of protein nature have been isolated from broth cultures of pneumococcus Type I by ultrafiltration, precipitation at a definite hydron concentration, and the separation of a soluble picrate fraction The method appears to be suitable for the initial purification of this antigen

Kasavin, J, and Grabfield, G P Blood Sugar Curves in Epidemic Encephalitis Arch Int Med, January, 1926, *Vol.* 86, 102

Twenty four blood sugar curves were studied in seventeen cases diagnosed as epidemic encephalitis or its sequelae

The curves were found to vary from the normal with such frequency that it seems probable that there is a fundamental disturbance of the sugar metabolism in this disease and during its mental sequelae

Feinberg, S M, and Lash, A F Blood Calcium in Eclampsia Surg, Gynec and Obst, February, 1926, p 255

One of the many theories advanced as to the etiology of eclampsia ascribes this condition to hypocalcemia Feinberg and Lash report their studies on this subject

Method—The determination of calcium was done according to the method of Kramer and Tisdall Blood was drawn from the arm and the serum separated Whenever possible 5 cubic centimeters of serum were used in the determinations To the serum in a 15 cubic centimeter centrifuge tube was added one-half its volume of a 3 per cent solution of ammonium oxalate This was allowed to stand until the following day The sides of the tube were then rubbed with a rubber tipped glass rod The tube was centrifuged at high speed for about ten minutes, the liquid carefully decanted, distilled water added, and centrifuged again This washing process was repeated three times To the washed sediment were added 5 cubic centimeters of normal sulphuric acid and the tube kept at a temperature of 75° C

This solution was titrated with a one-hundredth normal solution of potassium permanganate. The end point was considered that point at which a faint pink remained over fifteen seconds. The calculations to be used are based on the fact that each cubic centimeter of permanganate solution represents 0.2 milligram of calcium.

The average blood calcium in 11 cases of normal pregnancy was 10.94 mg per cent.

In 12 cases of eclampsia and preeclampsia, the average value was 10.21 mg per cent.

In 4 cases of various conditions simulating eclampsia (uræmia, chronic nephritis, epilepsy, and cavernous sinus thrombosis), the average value was 9.55 mg per cent.

It is concluded that, despite the theoretical decrease in calcium to be expected in eclampsia, there is no appreciable relation between the blood calcium and eclampsia.

Murphy W P. An Easy Method of Estimating the Amount of Jaundice by Means of the Blood Serum. *Boston Med and Surg Jour*, Feb 18 1926, xciv, 297.

Murphy prepares a set of standards for the estimation of the icterus index thus eliminating a colorimeter.

The dilutions of the potassium bichromate standard and the equivalent "index" figures follow:

1 10 000 = 1	1 500 = 20
1 5 000 = 2	1 400 = 25
1 2,000 = 5	1 200 = 50
1 1,000 = 10	1 133 = 75
1 066 = 15	1 100 = 100

Mueller J H. A Chemical Study of the Specific Elements of Tuberculin. *Jour Exper Med*, January, 1920, xlii, 1.

A study concerned with the protein or nonprotein nature of the substance in tuberculin to which is due its specifically toxic reaction in the body of the tuberculous animal.

A satisfactory conclusion as to the chemical nature of the fraction in question has not been formulated, but certain points in connection with the chemistry of "old tuberculin" have been demonstrated. It is concluded as a result of the studies made, that the specific precipitin reaction and the skin reaction given by old tuberculin are attributable to two separate substances present in this material. The cause of the precipitin reaction is a non-protein gum. It is also suggested that methods for the standardization of tuberculin by precipitin or complement fixation reactions should be revised.

In a second paper (The Preparation of Residue Antigens from Old Tuberculin. *Jour Exper Med*, January 1926, xlii, 9) Mueller describes the preparation in detail of the nonprotein gum responsible for the precipitin reaction. This material has been isolated from broth filtrates of human tubercle bacilli. It fixes complement and precipitates in high dilution in the presence of homologous immune serum but fails to give a skin test in tuberculous animals.

The authors have studied this question in passively immunized dogs

In dogs anaphylactic and immune sera usually gave approximately the same precipitin titer

If a normal dog after exsanguination, is transfused from an anaphylactic donor, the animal becomes typically hypersensitive

If the same procedure is followed except that the donor is an immunized animal, passive hypersusceptibility does not occur

It is concluded, therefore, that in the dog the sensitizing antibody and the immune antibody apparently have wholly different physiologic properties

Paullin, J E Glucose Utilization in Renal Glycosuria Trans Assn Am Physicians, 1925 *vol.* 131

A study of the results of the administration of glucose on the respiratory quotient, total metabolism, blood sugar, and glucose excretion in four cases of renal glycosuria

In three cases 100 gm were administered regardless of weight, in one case 175 gm per kilo

In all instances the response was similar to that of the normal individual and it would seem, therefore, that individuals with renal glycosuria metabolize and store carbohydrate the same as normal individuals and, so far as the evidence points, these patients do not develop diabetes mellitus

Goldzieher, M, and Peck, S M Granuloma Inguinale, Studies on the Etiology and Pathology Arch Path and Lab Med, April, 1926, *vol.* 1, 511

Studies conducted on seven cases are reported together with a detailed description of an organism isolated from all cases—*B. venerogranelulomatis*—producing complement fixing bodies, allergic tests, and granulomatous lesions in rabbits

The organism grown is illustrated in seven microphotographs and minutely described

The organism grows best at 37° C, but growth will proceed at room temperature Growth is most luxuriant under aerobic conditions, but occurs anaerobically

Sugar Reactions—Dextrose, galactose, inulin, lactose, maltose and saccharose are acidified with the production of gas The strains vary in their acid and gas producing qualities

Hemolysis was absent, no indol production occurred, gelatine was liquefied, milk coagulated, with acid production

On 4 per cent maltose peptone agar, plate cultures at 38° C, there are seen fine surface colonies in from twenty four to forty eight hours Some strains show no growth until they are forty eight hours old All strains reproduce themselves abundantly in twenty four hours on subculture On solid mediums there are formed small, round, transparent discs having a bluish opalescence, coalescing in older cultures The periphery of the colonies is homogeneous, while the center shows a fine granulation Confluent colonies have a wavy border In reflected light the colony is gray, but the center of older ones shows a yellowish color and a loss of transparency The sticky, mucoid growth, so characteristic of *B. mucosus*, was not observed

The cultures have a peculiar, sour, fetid odor, like that produced by the lesions

The growth characteristics on blood agar and rabbit blood agar do not differ from those observed on maltose peptone agar There is no hemolysis

Plain and glucose broth become diffusely clouded at the end of from twenty four to forty eight hours Later the growth settles to the bottom of the tube, forming a whitish sediment In older cultures it assumes a stringy quality and adheres somewhat to the sides of the tube There is no surface pellicle

On nutrient and asetic agar, growth differs in no way from that described

On potato, a heavy gray smear is formed, which later becomes brown and colors the edge of the potato black

The typical forms are best seen in smears from a twenty four to forty eight hour growth on Sabouraud's medium The organisms are from 1 to 6 microns in length, the

smallest resemble cocci, diplococci, or small bacilli, the larger show a bacillus form that tapers to a point at one end, and contain one two, or three granules. The large bacillus forms which we consider most typical are gram negative. The smaller forms especially the coccoid and coccobacillus types are sometimes gram positive. The inclusion bodies in the large forms are also many times gram positive. The staining reaction does not depend on the culture medium, nor could any other factor apparently be found to explain the remarkable pleochromism.

The inclusion granules are different from the so called polar bodies or any other known intracellular granules. They cannot be stained by special polar body staining methods, such as Abbott's and Moeller's. They do not show metachromatic staining. They are probably intensely stained parts of the bacillar substance, forming two or three round inclusions, often connected with a fine axial thread. Some of the bacilli contain but one coccoid body showing frequently a fine tail like process. The body of the bacillus is often so faintly stained that it surrounds the darker staining granules like a halo.

Capsules are not formed. Pleomorphism is extreme. Spores or pigment are absent.

The cultures often have a peculiar odor like that of rancid fat or sour sweat due to the presence of fatty acids and neutral fats.

Leendertz G. The Conduct of the Protein Corpuscles as a Reflection of Certain Diseased Processes in the Human Organism. Klin Wchnschr Jan 29 1926 v 175

Whatever the theories may be to explain the changes in the plasma and serum characterizing those diseases associated with protein destruction, the fact remains that increased protein destruction is accompanied by an increased lability of the globulin compounds.

A method is described for measuring the proportion between the coarsely dispersed or labile globulin and the degree of stability of the plasma and serum.

The refraction exponent of the serum is first determined with a Pulfrich refractometer.

One c.c. of serum and 10 c.c. of freshly prepared 0.025 per cent acetic acid solution are placed in a centrifuge tube graduated at its tip at 0.5 c.c.

The labile globulins flocculate. The tube is centrifuged for ten minutes at 3000 r.p.m.

The humpid liquid is carefully removed to the 0.5 c.c. mark. The pipette must not touch the walls of the tube, the fluid being saved.

A further 1 c.c. of serum is added and carefully stirred with the precipitate by means of a thin glass rod taking care to include the globulin fractions on the walls of the tube. As soon as the solution is entirely clear one drop is placed on the prism of the refractometer and the refraction index (R) read.

During this time 1 c.c. of serum is mixed in a test tube with 0.5 c.c. of the liquid removed from the globulin sediment and the refraction index of this solution determined (R_1).

The difference between R and R_1 multiplied by the dilution (15) is the numerical expression of the precipitable globulins in 1 c.c. of serum.

To find the measure of the stability of the serum the quotient is divided by the general protein content (R_5), this quotient representing the percentage of labile globulins in the general protein content of the serum.

$$\frac{(R - R_1) \times 10}{R_5} = \frac{Q}{100} \text{ gives}$$

$$Q = \frac{(R - R_1) \times 10}{R_5}$$

Or as the author suggests

$$\frac{\text{Labile globulin}}{\text{Serum protein}} = \text{quotient}$$

McJunkin F. A. Identification of Three Types of Mononuclear Phagocytes in the Peripheral Blood. Arch Int Med December 1925 xxxv, 799

An investigation of the mononuclear phagocytes classified as "large mononuclear leucocytes" or "transitional cells."

The investigation was undertaken, first, to determine the occurrence in the blood of hemendothelocytes and lymphendothelocytes, and second, the relationship of these two phagocytes to the mononuclear benzidin reacting phagocytes of human blood.

METHODS

Method A Phagocytosis in Vitro—To 2 cc of 3.8 per cent sodium citrate solution in a graduated centrifuge tube, 3 cc of blood is added. The citrated blood is mixed with 1 drop of India ink (Higgins') and the mixture incubated at 37° C for ten minutes. The mixture is centrifugated at low and then at high speed and the tube, after the careful complete removal of the supernatant liquid, returned to the incubator for fifteen minutes. The leucocytic layer is removed with a capillary pipette and smears are made on slides. It is essential to spread out completely the droplet and to guard against the smear reaching the edge of the slide, otherwise, most of the leucocytes may be lost. The phagocytosis obtained with human blood is quite complete but is very much less satisfactory in the case of the guinea pig and the rabbit.

Method B Peroxydase Staining of Smears with Benzidin—The fresh preparation of the leucocytic layer is covered for from thirty to sixty seconds with about 10 drops of an alcoholic solution of benzidin consisting of 100 mg of dry benzidin dissolved in 25 cc of acetone free 80 per cent methyl alcohol that contains 1 drop of hydrogen peroxide. Ten drops of distilled water are added to the alcoholic solution and two minutes are allowed for the reaction to take place. In the case of guinea pig or rabbit blood the reaction requires from five to ten minutes. Smears of guinea pig or rabbit blood must be treated as soon as they become dry, but the leucocytes of human blood react after several hours. The solution is washed off and hematoxylin (Harris' without acetic acid) applied for from twenty to sixty seconds. The hematoxylin is followed by 0.01 per cent eosin solution for twenty seconds. The preparation may also be stained by allowing Wright's stain, properly diluted, to act for from five to ten minutes.

Method C Peroxydase Staining with Benzidin in Paraffin Sections—The tissue, fixed for one day in 4 per cent formaldehyde solution, is cut into small bits not more than 2 mm in thickness, placed in 70 per cent acetone solution for two hours, pure acetone for thirty minutes, benzene for twenty minutes, and paraffin at 52° C for twenty minutes. Thin sections are attached to slides by allowing them to dry overnight at room temperature. The paraffin is removed with benzene (ten seconds) and acetone (ten seconds). The sections are covered with the diluted benzidin solution for five minutes, washed and stained with hematoxylin and eosin, as in Method B. After the eosin is washed off the excess of water is carefully removed with a soft cloth and the preparation dehydrated with acetone (ten seconds) and benzene (ten seconds), when it is mounted in balsam. To dehydrate in so short a time the solutions are run over the preparations from dropping bottles.

Method D Supravital Staining of Leucocytes with Neutral Red—In a centrifuge tube, to 10 cc of a neutral red solution consisting of 19 parts of saline solution and 1 part of saline solution saturated with neutral red (Gruebler), 2 or 3 drops of the fresh leucocytic layer without ink is added. After being mixed and allowed to stand for about ten minutes the cells are sedimented in the centrifuge tube and all of the supernatant liquid except about 2 drops is removed. With a platinum loop the cells are mixed with the liquid and a small droplet is transferred to the center of a cover glass rimmed with petrolatum and at once inverted on a slide for examination with oil immersion lens. The cells are permitted to remain in the citrate the shortest time possible.

Method E Zenker Formaldehyde Solution (Formol) Method of Mordanting the Supravital Dye in Smears—The reaction has usually reached the maximum intensity after about forty five minutes contact with the dye. Heavy smears are made and just as drying becomes complete they are dropped into a solution consisting of 5 parts of 40 per cent formaldehyde and 95 parts of Zenker's fluid without acetic acid. After two hours' fixation the preparations are washed and at once covered with hematoxylin (Harris' without acetic acid) for two minutes. The hematoxylin is washed off and the slides immediately blotted dry. The dye granules are well preserved, but the nuclear staining is faint.

Method F Iodine Vapor Method of Mordanting the Supravital Dye in Smears—Tissot cultures stained supravitaly have been successfully fixed in iodine vapor for microscopic examination and photographic purposes by Lewis. The smears of the fresh leucocytic layer, as they become dry, are placed for thirty minutes above iodine crystals in a glass jar 4 inches (10.1 cm) in diameter and 6 inches (15.2 cm) high with a ground glass cover. They are removed from the iodine vapor, covered with hematoxylin (Harris' without acetic acid) for one minute, washed and at once blotted dry with filter paper. The nuclei are well stained with hematoxylin, but the dye granules are apt to range from brown to black. Lewis does not mention using a nuclear stain.

Method G Zenker Formaldehyde Solution (Formol) Method of Preserving Supravital Stains in Paraffin Sections—Under anesthesia or immediately after removal from the living body a saline solution saturated with neutral red (Gruebler) is injected into the lymph nodes, spleen, or liver until the tissue becomes distended by the liquid. A second injection after a few minutes is advisable. After a half hour the tissue is placed for twelve hours in Zenker formaldehyde solution (formol) consisting of 15 cc of 40 per cent formaldehyde and 85 cc of Zenker's fluid without acetic acid. It is then cut into pieces not to exceed 3 mm in thickness and transferred to Zenker's fluid without acetic acid for from twelve to twenty-four hours. The bits of tissue are then placed in pure absolute acetone for one hour (two changes), in benzene for twenty minutes and in paraffin at 52° C for twenty minutes. Extra thin sections are then attached to slides with albumin fixative by allowing to dry overnight at room temperature. To stain, the paraffin is removed with xylene (ten seconds) and pure acetone (ten seconds). After immersion in water (five seconds) the slide is stained very lightly with hematoxylin (Harris' without acetic acid) for about five seconds. The section is then dehydrated with pure acetone for ten seconds, at once covered with xylene for ten seconds and mounted in balsam. To limit the action of the acetone and xylene to these times, the slides are stained singly and the solutions run over them from dropping bottles.

CONCLUSIONS

1 Mononuclear benzidin positive phagocytes reacting to neutral red supravital staining are present in normal human blood, occasionally in guinea pig blood, but not in rabbit blood.

Since they are found only in bone marrow and spleen they probably originate in these tissues.

2 Lymphendotheliocytes benzidin negative mononuclear phagocytes reacting to supravital staining with neutral red (occasionally with the formation of granules in rosettes), are present in the normal peripheral blood of guinea pigs, rabbits and man. They arise from the lymphatic reticuloendothelium and are transformed into the epithelioid cells of tubercles.

3 Hemendotheliocytes benzidin negative mononuclear phagocytes not reacting to supravital staining with neutral red probably arise from the blood vascular endothelium and do not occur in the normal peripheral blood.

McCrackan, R. F. and Passamaneck, E. Manganese in the Urine, its Detection and Determination. Arch. Path. and Lab. Med. April 1926: 585.

To 100 cc of urine in a Kjeldahl flask, 20 cc of concentrated nitric acid is added. If the urine contains less than 1 mg of manganese per liter of urine, larger amounts in the same proportion are used. This is followed by evaporation to a paste on a sand bath, after cooling, 5 cc of concentrated sulphuric acid is added. The preparation is then heated at high temperature until about a third of the acid is driven off as heavy white fumes. With urine high in phosphates, or when a large quantity of urine is used for the analysis, the amount of sulphuric acid may have to be increased. After cooling, 5 cc of concentrated nitric acid is added, followed by heating until brown fumes disappear. If oxidation does not seem to be complete, this step is repeated again and again until there is no doubt more sulphuric acid being added if necessary. After cooling, transfer is made to a 100 cc volumetric flask by means of about 75 cc of distilled water. Then 5 cc of concentrated nitric acid, 1 cc of tenth normal silver nitrate and 1 cc of 50 per cent ammonium persulphate are added and diluted to the mark. One or more standards are prepared with similar

amounts of reagents from manganous sulphate or manganous nitrate, or potassium permanganate in 100 cc flasks, and both the known and the unknown are heated at the same time in the water bath until the formation of permanganic acid is complete. If the depth of color in the unknown is deep enough, comparison is made in the colorimeter, or in Nessler tubes if it is too faint. When manganese is found a blank test should be made on the reagents as a control.

In case the acidity is already quite high due to using more than the amount of sulphuric acid recommended, less nitric acid may be used before adding the persulphate, or its use may be omitted. In case much silica from the glassware is present, it may be ignored until after the solution is made up to volume and the color developed. Then it may be removed from a portion by centrifuging, or it may be allowed to settle by gravity.

The test is so sensitive that great care must be taken to prevent contamination.

The amount of manganese in one normal urine has been found to be less than 1 part in 50,000,000.

Jaffe, R. H. **Studies in Vital Staining in Experimental Tuberculosis**. *Am Rev Tuberc*, February, 1926, *vol*, 97.

Most intensive vital staining of rabbits with India ink or colloidal iron does not prevent the formation of tubercles. This observation is not in accordance with the possibility of a functional elimination of cells by the granular storage of foreign material.

The decrease of the dye in the growing tubercle, and its absence under certain conditions in larger tubercles, is due to lack of proper circulation rather than to diminished vitality of the epithelioid cells.

Schuback, A. **The Bacteriologic Investigation of Material Containing Proteus**. *Klin Wehnschr*, January 8, 1926, *v*, 67.

For the culture of material containing *B. proteus*, Schuback uses nutrient agar to each 10 cc of which has been added 0.2 to 0.3 cc of proteus agglutinating serum diluted 1:1 with 0.5 per cent phenol. The serum is prepared by the immunization of rabbits and should be of not less than 1:6,000 titer.

By this procedure the tendency of proteus organisms to overrun poured plates is inhibited.

Balint, M. **Buffered Water for the Romanowsky Giemsa Stain**. *Klin Wehnschr*, January 22, 1926, *v*, 147.

After the blood smears have been air dried they are fixed for five minutes with Jenner's solution and prestained for ten minutes by the addition of 2 cc of the following solution:

KH_2PO_4	9.078 gm
$\text{Na}_2\text{HPO}_4 \cdot (2\text{H}_2\text{O})$	11.876 gm
Boiled distilled water	100 cc

The smears are then washed with this buffered water, stained for twenty-five minutes with Giemsa (1 drop to 1 cc of buffered water), washed with buffered water and air dried.

Chanutin, A., and Guy, L. P. **The Fate of Creatine When Administered to Man**. *Jour Biol Chem*, January, 1926, *lxvii*, 29.

As a result of careful experiments on two normal individuals the following conclusions were formulated:

1. The absorption of creatine from the alimentary tract appears to be complete. There is no evidence of its bacterial decomposition.

2. The creatinine content of the urine in man increases after the ingestion of large doses of creatine, the increase, apparently, being derived directly from the creatine fed.

3. Evidence is presented to indicate that creatine has an indirect action on nitrogen metabolism.

Haynes B. Modification of the French Azure C Tissue Stain Stain Technology April, 1926, 1, 68

- 1 Xylol, 3 min
- 2 Absolute ethyl alcohol, 3 min
- 3 Ninety five per cent ethyl alcohol, 3 min
- 4 Water 3 min
- 5 One and five tenths per cent aqueous solution Azure I, 5 min
- 6 Absolute ethyl alcohol, 5 to 10 seconds (For formalin fixed material use 3 per cent glacial acetic acid in absolute alcohol)
- 7 Saturated solution alcohol soluble eosin (ethyl eosin) in clove oil, 30 seconds
- 8 Xylol, 10 to 30 seconds
- 9 Xylol, 12 min
- 10 Xylol, 12 min
- 11 Xylol balsam

Young C C and Orr P F Dosage of Toxin for Active Immunization against Scarlet Fever Jour Am Med Assn, May 1 1926, lxxxvi, 1340

A preliminary report of an attempt to determine whether as great an immunity can be produced with three as with five doses of scarlet fever toxin

The administration of three doses in a group of twenty four individuals—500, 5 000 and 30,000 skin test doses—at intervals of two weeks was found to be without injurious effects and to produce a satisfactory immunity as great or greater than that following five doses as usually advised

Welker W H. Thomas W A. and Hektoen L Urinary Proteins Crystalline Proteins of Nephritis Jour Am Med Assn May 1 1926 lxxxvi, 1333

In ten cases of nephritis proteins were obtained in the form of globular crystals from the urine In one instance the protein appeared as needle like crystals

Precipitin tests indicate that the protein crystals consist of compounds of serum albumin, globulin and pseudoglobulin

As a preliminary treatment, the urine was saturated to 25 per cent with ammonium sulphate, left standing for a few minutes until flocculation had taken place, and then filtered. The filtrate was returned to the filter paper until a clear fluid was obtained, which was completely saturated with ammonium sulphate. The precipitate was dissolved with the requisite amount of distilled water giving a protein solution of moderate concentration. This solution was treated with saturated ammonium sulphate solution until a slight, but permanent, flocculent precipitate formed. The mixture was then filtered and refiltered until a crystal clear fluid resulted. The solution was set aside in a crystallizing dish and usually at the end of twenty four hours a copious sediment had appeared which under the microscope was composed of globular crystals of protein as shown by appropriate tests. After standing for a number of days but before any gross separation of ammonium sulphate had occurred the solution was filtered the sediment on the filter paper dissolved in distilled water and again treated with saturated ammonium sulphate until a slight but permanent precipitate had formed. The solution was filtered and refiltered until clear and again set aside for crystallization. Again globular crystals of protein appeared at the end of twenty four hours.

Noguchi H. Abnormal Bacteria Flagella in Cultures Their Resemblance to Spirochetes Jour Am Med Assn, May 1, 1926 lxxxvi, 1327

Besides the leptospira like filaments, probably originating from red blood corpuscles under certain conditions in vitro other spiral elements exist which may be erroneously interpreted as spirochetes. These are the exaggerated detached flagella of certain bacteria produced under cultural conditions. In cultural studies of microorganisms the occurrence of

these spiral elements must be borne in mind, particularly in connection with dark field illumination

Spirochetes belonging to the spirochaeta and treponema groups also produce, under certain cultural conditions, exaggerated flagellar appendages. These terminal flagella are similar in appearance and structure to the axial spiral filaments of the same organisms, but are much finer. The axial filaments are covered with a layer of cytoplasm which can be removed by the action of bile. The motility of these organisms resides in the portion of the filament next the attachment of the flagellum at either end.

The great resemblance which exists between the flagella of motile bacteria and the flagellar and axial spiral apparatus of certain spirochetes seems to indicate that the axial filaments are probably a modified apparatus of similar origin especially adapted to the locomotion of spirochetes, and therefore supports the hypothesis of a close phylogenetic relationship between bacteria and spirochetes.

The paper is illustrated with twenty five microphotographs.

Adams, S. F., and Brown, G. E. The Blood in Cases of Hypertension. The Relationship between Anemia and Renal Insufficiency. *Ann. Clin. Med.*, December, 1925, iv, 463.

In 76 cases of hypertension uncomplicated by gross loss of blood or by conditions obviously related to the production of anemia and in which renal function was adequate, the hemoglobin was more than 100 per cent in 60 per cent.

In 90 per cent of cases where renal function was inadequate the hemoglobin and red cells were diminished.

A parallelism seems to exist between erythrocytic and renal function in cases of hypertension. The recovery of the blood does not parallel the recovery of renal function.

The presence of anemia in cases of primary hypertension and consequent arteriosclerosis, in the absence of complicating disease or gross loss of blood is good evidence of existing or preexisting renal insufficiency.

Felty, A. R., and Heatley, C. A. The Nasal Passages in Lobar Pneumonia. *Jour. Am. Med. Assn.*, April 17, 1926, lxxvi, 1195.

In a series of sixteen cases of lobar pneumonia, pneumococci of corresponding type to those found in the sputum were isolated from the middle fossae of the nose in every case.

The nasal passages in fifteen of the sixteen patients were examined with the nasopharyngoscope, of these, all showed hyperemia of the mucous membranes, and six had signs of acute suppurative sinusitis. In eight cases, anteroposterior roentgenograms of the sinuses were made, in seven patients, clouding of one or more of the sinuses was observed.

In this small series of patients, acute pneumococcal sinusitis was a frequent accompaniment of lobar pneumonia.

Lindsay, J. W., Rice, E. C., and Selinger, M. A. Scarlet Fever. *Jour. Am. Med. Assn.*, April 17, 1926, lxxvi, 1191.

As a result of an analysis of cases of scarlet fever studied in the Garfield Memorial Hospital the following conclusions are presented:

- 1 The intracutaneous injection of properly concentrated scarlet fever toxin, after the method of the Dicks, properly controlled by use in groups, is a reliable means of determining susceptibility or immunity to scarlet fever.

- 2 This method is also frequently of definite assistance in diagnosis.

- 3 It is possible actively to immunize susceptibles for a considerable period.

- 4 Care should be used to adjust the dose of toxin for active immunization so as to avoid serious reactions in those who may have an unusually small amount of natural anti-toxin, as indicated by an unusually large and intensely positive Dick reaction.

- 5 There is ample evidence that the serum prepared by the method of Dochez, now available for the treatment of scarlet fever, possesses true and specific antitoxic properties in effective concentrations.

6 The early use of sufficient amounts of antitoxin apparently reduced the incidence of complications of severe degree

7 A single large dose of antitoxin will probably prove more satisfactory, from every standpoint, than a small or moderate dose with the possibility of repetition

8 The intramuscular injection appears to be satisfactory in practically all cases, the intravenous possibly being indicated in critical conditions

9 We suggest the advisability of testing all patients for sensitiveness to serum before giving the antitoxin, even though workers are not in complete agreement as to the importance of this procedure

Herrold R D and Saelhoff C C Skin Reactions with Filtrate of Koch Strain of *Bacillus Tuberculosis* Jour Am Med Assn March 13 1926 lxxvi 747

The so called Koch strain of the tubercle bacillus is an avirulent culture which gives a profuse growth overnight on the ordinary solid and fluid nutrient mediums

Transplants from solid medium were made into nutrient broth containing 0.1 per cent dextrose and 0.1 per cent dibasic sodium phosphate in place of the sodium chloride of the ordinary broth. A four day growth was passed through Berkefeld N filters and the filtrate diluted with physiologic sodium chloride solution for skin tests. Skin reactions were obtained in normal adult persons in dilutions up to 1:50 injected intracutaneously in a quantity of 0.1 cc. Tuberculin syringes were used and a 27 gauge needle. The injections were made in the forearm as a rule but in persons with a thin skin the arm was satisfactory.

Within twenty four hours the majority of apparently normal adult persons developed an area of redness at the site of injection which varied from 1 to 3 cm in diameter and reached its maximum in from twenty four to forty eight hours after fading there frequently is left a pigment spot which persists for several days and in some instances for several weeks. Such reactions are classified as positive.

A second type of reaction was smaller in size—from 5 to 10 mm in diameter—and after forty eight hours disappeared without leaving any pigmentation. This reaction is considered as doubtful.

The tests without reaction at the end of forty eight hours are classed as negative. Controls of broth diluted with salt solution gave no reactions.

The accompanying table shows types of patients tested.

TYPE OF PATIENT	NUMBER OF PATIENTS	NUMBER OF REACTIONS		
		POSITIVE	DOUBTFUL	NEGATIVE
Advanced tuberculosis	35	0	0	35
Moderately advanced tuberculosis	31	0	0	31
Incipient tuberculosis	8	0	0	8
Apparently normal adults	61	47	8	6
Apparently normal children	14	2	2	9

Obviously, the interpretation of these results must be tentative, but the presence of a reaction in the majority of apparently normal adult persons seems to indicate that there is a substance produced by the growth of this strain of tubercle bacillus in broth which acts in a different way from tuberculin. The reaction to the filtrate in normal adult persons and the absence of reaction in tuberculous patients and in many children may mean that in the presence of an active tuberculous focus there may be sufficient antistuberculous substance produced to neutralize the toxic substance.

Smith D C and Gill R D Nonspecificity of the Justin Test Am Jour Syph, April 1926 ix 2

After a study of the test in 17 syphilitics and 68 nonsyphilitics it was concluded that the Justin test is nonspecific and unreliable as a diagnostic procedure.

Poire, A F, and Carianza, M A Staining Methods for the Koch Bacillus *Semana med*, October 8, 1925, *xxxx*, 877

The authors, after a comparative study of various methods, recommend the following

- 1 Fix slowly by heating, at a temperature not exceeding 60° to 70° F
- 2 Pour on the staining agent, drop by drop of carbolized fuchsin, until the entire specimen is covered
- 3 Heat slowly until some vapor is given off
- 4 Leave the specimen alone until the disappearance of vapors
- 5 Heat again, until vapors are given off
- 6 Repeat the fourth item
- 7 Repeat the fifth item
- 8 Repeat the fourth item
- 9 Pour cold water on the specimen
- 10 Wash with sufficient water under the water faucet, not too violently
- 11 Decolorize with sodium sulphite 10 per cent, heated to 80° and newly prepared, which decolorizes in a few seconds
- 12 Wash with an abundance of water
- 13 Stain the background with methylene blue in dilute watery alcoholic solution, in a proportion of one drop per five cubic centimeters of distilled water
- 14 Wash with an abundance of water
- 15 Slant the specimen, so as to permit the fluid to drain off Dry with filter paper then gently heat the slide Examine

The sodium sulphite used by the authors is crystallized and preserved in well closed bottles, in order to guard against its transformation through the presence of air The solutions are prepared just before using, because after twenty four hours, the sulphite becomes changed and transformed into the sulphate, its power of decolorization diminishing until after three days it ceases to decolorize The quantity that is prepared should be proportionate to its daily employment, and it is therefore advisable to make packages of two and five grams, which are kept in well closed wide necked flasks, ready to be dissolved in 20 or 50 cc of distilled water

Patton, H W, Blackford, S D, and Smith, D C Cutaneous Tests with Suspensions of *Treponema Pallida* *Med Jour and Rec*, January 6, 1926, *xxxx*, 4

A study of 100 syphilitic and 60 nonsyphilitic patients from which the following conclusions are formulated

- 1 Intradermal injections of saline suspensions of *Treponema pallida* are without value as a practical diagnostic test
- 2 A suspension of *Treponema pallida* in twentieth normal sodium hydroxide gives no better results than the saline suspension
- 3 Potassium iodide in doses of 60 grains daily does not affect the results of intracutaneous tests with either solution

Moritz, A R The State of the Serum Calcium in Experimental Hypo- and Hypercalcemia *Jour Biol Chem*, December, 1925, *lvi*, 343

In a general way, a decrease in serum calcium following thyroparathyroidectomy shows a disproportionately great decrease of the diffusible fraction, but in an increase in serum calcium produced by injection of parathyroid extract (Collip) the ratio of colloidal and diffusible calcium did not show consistent changes

Regan, J C, and Tolstouhov, A Significance of the Blood Chemical Changes in Pertussis *Jour Am Med Assn*, April 10, 1926, *lxxvi*, 1116

A total of 682 blood chemical analyses performed in cases of pertussis have given the following results

- 1 There is a diminution of the total inorganic phosphorus associated with a lowering of the hydrogen ion concentration of the blood, while the plasma bicarbonate remains within normal limits

2 These changes occur early in the disease appearing in the case of the inorganic phosphorus in the catarrhal stage

3 Both alterations are well developed, especially the change in phosphorus, during the first few weeks of the paroxysms, and show a certain degree of parallelism in their course which signifies a close interrelation

4 In moderate and severe cases treated with alkalis the inorganic phosphorus rises steadily from the third week, while in untreated cases of the mild type, the rise does not begin until the sixth week. The same is true in a less decided way of a P_{H_2} value before, as compared to those during and after, treatment

5 The diminution of inorganic phosphorus bears no relation to age, but only to the stage of the disease, and for reasons mentioned in the text has no underlying rachitic basis

6 The calcium content, while exhibiting slight mobility, as the result, possibly, of shifting of calcium in connection with the characteristic phosphorus and P_{H_2} alterations, has no constant alterations of a distinct type

7 These changes indicate an acidosis of an uncompensated type (Type 6, Van Slyke), which has as a cause the accumulation or increased concentration of free carbon dioxide in the blood. This laboratory observation is easily correlated with several of the symptoms so prominent in pertussis—the paroxysms, the vomiting, parenchymatous emphysema and convulsions

8 The vomiting of the disease may be a compensatory mechanism adopted by the body to eliminate acid in an attempt to maintain a normal acid base balance

9 This contention of an uncompensated acidosis is further substantiated by the effects on the disease of alkali therapy

10 Alkalis administered early appear usually to abort the disease, and associated with the cure is a rapid rise of inorganic phosphorus and a change in P_{H_2} of the blood, while, if given late, cure supervenes in a relatively short period

CONCLUSIONS

There occurs in pertussis an uncompensated acidosis which is intimately connected with the pathogenesis of the paroxysms

If the acid base unbalance is corrected the clinical symptoms are quickly ameliorated, and the organism returns to normal

Sheridan W F Rapid Paraffin Embedding of Tissue Internat Assn Med Mus Bull
May 4, 1926, xi, 124

Salt mouth bottles of 200 c.c. capacity are loosely packed with a layer of filter or tissue paper and filled with acetone so as to provide a layer about one half to three quarters of an inch above the level of the paper. The pieces of tissue should not be over 3 mm. in thickness

- 1 Boil tissue in neutral 10 per cent formalin for thirty seconds
- 2 Blot.
- 3 Acetone two and one half to three hours
- 4 Blot
- 5 Xylene fifteen to twenty minutes or until tissue is translucent.
- 6 Blot
- 7 Paraffin 52° C melting point 4 twenty minute changes in 60° C oven
- 8 Embed and congeal with cold water

Sheridan W F Rapid Hematoxylin Eosin Staining Method for Paraffin Sections Internat Assn Med Mus Bull, May 4, 1926 xi 57

The sections should not be over 5 microns in thickness. Attach the sections to the slides with Mayer's glycerin albumin and stain as follows

- 1 Xylene two minutes
- 2 Rinse with alcohol (90 per cent) from drop bottle

- 3 Hematoxylin (Harris) two minutes
- 4 Rinse with alcohol (95 per cent) from drop bottle
- 5 Acid alcohol (1 per cent HCl in 70 per cent alcohol) fifteen or more seconds
- 6 Rinse with alcohol (95 per cent) from drop bottle
- 7 Ammoniated alcohol (stronger ammonia water 4 drops, alcohol 95 per cent 50 mls) until rose color is replaced by blue—about one minute
- 8 Rinse with alcohol (95 per cent) from drop bottle
- 9 Eosin (eosin alcohol soluble 0.25 per cent in alcohol, 95 per cent) thirty seconds
- 10 Rinse with acetone from drop bottle
- 11 Xylene
- 12 Mount in balsam

The use of Harris' hematoxylin is essential for good results. The ammoniated alcohol should be prepared just before use and the rinsing with acetone should be thorough. The acetone should not give more than a faint turbidity on shaking with xylol (i.e., nearly water free).

The clearing in xylol after acetone is almost instantaneous.

Pierce, L. H. A Rapid Stain for Nerve Tissue. Internat. Assn. Med. Mus. Bull., May 4, 1925, vi, 55.

Fuchsin f. bae (Gruber) saturated aqueous solution.....	gtt 35
Erythrosin (Gruber) 1 per cent aqueous solution.....	gtt 15
Methyl orange (Campbell and Bell) saturated aqueous solution.....	gtt 40
Aniline blue porous (Campbell and Bell) saturated aqueous solution.....	gtt 25

Mix in the order listed and permit to stand for twenty-four hours. Frequent agitation of the solution during this period is desirable as more perfect blending results. No precipitates are formed. Since the mixture appears to stand up well, multiples of the unit above may be mixed. Three or four times the unit will provide sufficient mixture for many slides. A dropper is preferred for accuracy in mixing.

The prepared slide is removed from the water and the excess shaken off. Sufficient stain to cover the section is added and permitted to act for ten to thirty seconds. Wash in running water, dehydrate in absolute alcohol (alcohol that has been dehydrated with anhydrous copper sulphate will serve the purpose and is much less expensive), blot carefully but quickly, clear in xylol and mount in balsam.

It is recommended that several sections of the same specimen be stained over periods ranging from five seconds to sixty seconds at five-second intervals, in order that the reactions of the many elements to the stain may be noted. This study will prove of value and serve as a guide for future use in demonstrating some one particular feature by selecting the time suitable for showing the desired object to best advantage.

In general the nerve cells stain light to very dark purple with deeper staining Nissl bodies fairly well seen. Normal cells usually stain deeply in twenty seconds. The nuclei are quite definitely blue with an orange-red nucleolus. In certain cells the nucleus is outlined and transparent but the chromatin fragments stand out sharply in deep blue.

The myelin varies from pink to deep red. Particularly does the latter tint prevail if this substance is granular. The axon cylinder is almost black, but reacts less intensely in proportion as it has degenerated until it takes the orange tint. This remark applies equally to the nerve cells which become mottled if degenerating and finally react to orange staining exclusively.

The neurolemma is well shown as a light or dark blue tracery, depending on the duration of staining.

The neuroglia likewise reacts to the blue stain but shows quite well the detail within its structure if not overstained. The pathologic types are easily recognized.

The erythrocytes stain orange to red-orange in proportion to the time of stain contact. Lymphocytes stain a pale blue.

Corpora amylacea are pale blue.

An interesting and practical step in the technic consists of treating the section as it is taken from the first alcohol with a 1 per cent solution of glacial acetic acid in 90 per cent alcohol for ten to twenty seconds or longer as the worker wishes. The watery stain is applied directly to the section after the excess of the acid alcohol has been shaken off. The staining period is shortened but may be carried on for the full limit. The observer will note marked intensity in staining in some of the elements and this may prove of value in certain instances. The nerve cell detail is obscured if too long staining is done. On the other hand the neuroglia is intensified as are all the connective tissues. The myelin is distinctly benefited by this treatment and all red cells stain a bright orange.

Kohn, L. A. Recurrent Type I Pneumonia. Jour Am Med Assn December 12 1913
lxxxv, 1888

Two attacks of Type I pneumococcus pneumonia occurred at an interval of six weeks in a person who had suffered from several previous untyped pneumonias. There was little response in the production of humoral antibodies to these attacks.

The first attack recorded was undoubtedly a lobar infection, and the isolation of pneumococci from the blood stream with the presence of organisms and abundant precipitogen in the sputum establishes it as due to Type I. The second attack while involving three separate lung areas in three lobes was nevertheless croupous pneumonia clinically and the patches of consolidation appeared by the roentgen ray to be larger than those usually involved in lobular pneumonia. The sputum was twice injected into mice and each time a pure culture of pneumococcus Type I was recovered. This organism was fatal to mice when injected intraperitoneally in quantities down to 10 cc of eighteen hour broth culture, and was agglutinated to the titer of Type I agglutinating serum (1:64). It is exceedingly unlikely that any other organism was responsible for the pneumonia.

Lundquist, B. A Proposed Modification of the Kaiserling Method for Preserving Gross Specimens. Internat Assn Med Mus Bull May 4 1916, vi, 16

SOLUTION I

Potassium acetate	- - - - -	85 gm
Potassium nitrate	- - - - -	45 gm
Chloral hydrate	- - - - -	80 gm
Formaldehyde (10 per cent gas)	- - - - -	444 cc
Water	- - - - -	4000 cc

The technic for using the solution is the same as for other solutions. Specimens are placed in the fixing fluid as soon as possible. Ten to twelve times the volume of fixing fluid to the volume of the specimens is used and the specimens are not allowed to lie against one another or against the bottom of the container. A container deep enough to permit suspension of the specimen by a string attached to a paraffined cork is used. Care must be exercised to attach the string in such a way that the suspended specimen will assume its natural shape. The use of the paraffined corks has the advantage of facilitating the removal of any specimen desired, and of allowing a larger number of specimens to be placed in one jar without the objectionable feature of one specimen pressing against another at the bottom of the jar. Specimens should be thoroughly fixed but should not be left in the fixing solution too long, beyond this point. However, the danger of loss of color due to overfixation is not nearly so great with this method as with the original Kaiserling method. The time of fixation of course, varies with each specimen. After fixation the specimens are thoroughly washed in running water to remove all formaldehyde. At this stage the specimens are trimmed and all specimens having cut surfaces are re surfaced. They are then placed in the following solution.

SOLUTION II

Potassium acetate	- - - - -	10 gm
Chloral hydrate	- - - - -	5 gm
Glycerin	- - - - -	10 cc
Water	- - - - -	90 cc

This solution is changed twice, the specimens remaining in the first solution about twelve hours. The colors become brighter in this solution and the consistency and color obtained are much closer to the original than by any other method tried. The selection of a preservative for the final mounting fluid to prevent the growth of yeasts, fungi, etc., is a very important factor in the preservation of the colors, as well as of the consistency of the specimen, and must receive still further study. There is much evidence that the use of arsenious acid for this purpose is preferable to formol, phenol or thymol, but this is still under trial.

Kohn, L. A. Acute Mercuric Chloride Poisoning. *Arch Int Med*, February, 1926, *xxxvii*, 225

Death from mercuric chloride taken by mouth may ensue within hours, apparently with circulatory collapse, with little renal damage and no evidence of uremia. Evidence is presented which suggests that direct myocardial damage may account in part, at least, for this early toxic death. In three severely poisoned cases, the white count reached 34,000 or higher in a few hours, and it is suggested that the degree of elevation of the leucocytes may be an index of the severity of poisoning, with an unfavorable prognosis when levels of from 30,000 to 40,000 are found. Last, while it is not denied that sodium thiosulphate may have value in treatment, it should be emphasized that it may fail to exert detoxicant action, and should not be administered to the neglect of established therapeutic methods.

Rosen, I., and Krasnow, F. Blood Cholesterol Findings in Syphilis and in Other Skin Diseases. *Arch Dermat and Syph*, April, 1926, *lxi*, 506

Report of a study of the blood cholesterol in a variety of conditions.

The blood cholesterol was low in 100 per cent of the patients with untreated primary syphilis, in 50 per cent of the untreated secondary cases, and in 25 per cent of the untreated tertiary cases.

After treatment all our primary cases showed a rise in the cholesterol content to normal or above normal, whereas some of the secondary and tertiary cases remained low.

The cholesterol content in 50 per cent of the pregnant syphilitic women showed a high cholesterol value, 35 per cent showed a normal value, and 5 per cent a low value.

The blood cholesterol content in two infants with active manifestations of syphilis was low.

The blood cholesterol content in 82 per cent of the treated patients who had congenital syphilis was normal.

There seems to be no direct connection, at least as far as these studies are concerned, between the cholesterol content of the blood and the results of the Wassermann reaction.

The cholesterol content of the blood was high in psoriasis and dermatitis venenata and normal in acne vulgaris, dermatophytosis and dermatitis seborrheica.

Berger, S. S., Cohen, M. B., and Sellman, J. J. Liver Functional Tests, a Comparative Study of Five Methods in 100 Clinical Cases. *Jour Am Med Assn*, April 10, 1926, *lxxvi*, 1114

All the tests were done either simultaneously or within forty eight hours, the following being studied: Van den Bergh's, Vidal hemoclastic, Rosenthal, examination of urine for urobilin and urobilinogen, and for bile salts.

In 10 cases of liver disease with jaundice due to stone or tumor the tests showed: Van den Bergh 100 per cent, Vidal 40 per cent, dye 60 per cent, urobilin 30 per cent, bile salts in urine 80 per cent.

With 6 cases of liver disease without jaundice: Van den Bergh 83.3 per cent, Vidal 50 per cent, dye 33.3 per cent, urobilin 66.6 per cent, bile salts in urine 33.3 per cent.

In 37 cases in which liver disease was suspected, the positive reactions were as follows: Van den Bergh 37.9 per cent, Vidal 59.4 per cent, dye 16.2 per cent, urobilin 32.5 per cent, bile salts in urine 48.6 per cent.

In 38 cases in which liver disease was not suspected the results obtained were Van den Bergh 43.1 per cent, Widal 33.4 per cent, dye 10.5 per cent, urobilin 5.5 per cent, bilirubin 2.6 per cent.

As a result of the studies the following conclusions are advanced:

1. It is important to bear in mind that these tests represent different functions of the liver. Any one or more or all of these functions may become impaired. Again one or more of these functions may escape injury. Therefore, the various tests do not give parallel results. When we attempted to separate clinical cases into groups of liver disease or no liver disease by means of any one of these tests unsupported by other clinical evidence, we were unable to do so.

2. When all the tests were positive, we were dealing with liver disease, clinically of the most severe type, namely toxic jaundice.

3. When all tests were positive except one, namely, four positive and one negative, clinical liver disease was present usually of a chronic type such as that seen in Bright's disease or pernicious anemia and cirrhosis.

4. In every case in which all the tests were positive except the Widal there was obstructive jaundice due to tumor. This finding is of great value in differential diagnosis.

5. When only three tests were positive it was impossible to correlate the findings with the clinical picture, as there were many cases in which liver disease was suspected which did not give positive reactions to more than one or two tests, and conversely there were many cases in which liver disease was unsuspected which gave as many positive results.

6. At present they are of use chiefly in the differential diagnosis and in following the progress of a given case. The greatest amount of information can be gained by doing all the tests simultaneously and repeating them often.

Wile N. J. and Belote G. H. Syphilitic Alopecia: Its Relation to Neurosyphilis. *Arch. Dermat. and Syph.* April, 1926, xiii, 495.

A histologic study of 77 cases. Two distinct types are recognized: that occurring without visible accompanying syphilides, and that in which the loss of hair is apparently due to papular or other lesions on the scalp.

The authors conclude that:

1. Syphilitic alopecia of the essential type has a high associated incidence of meningococcal syphilis, as indicated by spinal fluid findings.

2. The absence of the accepted criteria in the spinal fluid cannot, moreover, be accepted as absolute evidence of the absence of such involvement.

3. Microscopic study shows that the essential syphilitic alopecia is not due to any local pathologic disturbance of the scalp or more specifically of the follicular apparatus. It is therefore not a true syphilide.

4. Clinical analogy affords the suggestion that it is due to endocrine dysfunction as a result of association and involvement of the autonomic nervous system.

5. Symptomatic alopecia representing a smaller group of the entire syndrome is a true syphilide, apparently caused by a perifollicular plasmaoma.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

Modern Clinical Syphilology

IT IS obviously a matter of great difficulty—if, indeed, it can be said to be possible—to compress within the limits of a single volume of average size an adequate consideration of the intimate relation of syphilis to the practice of medicine and it is, therefore, inevitable, that any author contemplating a treatise upon syphilis must decide to whom his volume shall be addressed, the syphilographer or the practitioner at large.

Dr Stokes has chosen the latter audience for his presentation and the resultant volume constitutes one of the most practical and usable works upon syphilis that it has been the privilege of the reviewer to examine. This book is of immediate practical interest and value to all who are interested in syphilis from whatever angle, to the laboratory worker who must have a working knowledge of clinical syphilology to apply intelligently his laboratory studies to the particular case, to the syphilographer because of its thorough survey and presentation of the modern knowledge of this subject, and most of all, perhaps, to the practitioner who essays to treat this onerous infection.

It is not to be denied, as has been said, that the development of both the Wassermann test and the arsenicals has had a tendency to develop a clan of pseudosyphilographers for whom the Wassermann test in the commercial laboratory makes the diagnosis while a few "shots" of "neo" suffice to treat the disease.

When such books as that of Dr Stokes and his collaborators are available there is no excuse for pseudosyphilography.

After a brief discussion (26 pages) of the etiology, pathology, and immunology of syphilis, follow excellent and practical discussions of methods of clinical approach and physical examination excellently written and clearly illustrated.

Conveying a lesson, not always learned it would seem, there follows a clear discussion of the use and application of laboratory methods of examination which, it is emphasized, must be utilized and interpreted in conjunction with the other findings previously described.

Following this 138 pages are devoted to treatment—a discussion of the fundamental principles, a consideration of the uses of mercury, bismuth, the iodides and the arsenicals, and a clear, detailed and illustrated presentation of technique.

The remainder of the book is concerned with the diagnosis and treatment of syphilis in various stages and as affecting various organs and structures with final chapters on public health and miscellaneous aspects.

It is, of course, unnecessary to say that the book amply reflects an extensive and varied clinical experience.

It is throughout eminently practical, and specific. The illustrations are not only numerous and well reproduced but they *illustrate*. There are not only instructions as to what to do but clear directions, involving every step as to *how* to do it.

*Modern Clinical Syphilology. By John H. Stokes, Professor of Dermatology and Syphilology, University of Pennsylvania, with the cooperation of P. A. O'Leary and W. H. Golckermann of the Mayo Clinic and L. W. Shaffer and C. J. White of the University of Pennsylvania. Pp 1144. 865 illustrations and 366 figures. Cloth. Price \$12.00 net. W. B. Saunders Co. Philadelphia.

NOTE. Insofar as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

An excellent feature are the numerous schematic comparisons, resumés, tabular summaries, case discussions, and aphoristic summaries—such as “the seven bads” in relation to arsenic tolerance “the decalogue of dermatitis prevention” and the like which abound throughout the book

This is a book which can be unreservedly commended and which can be purchased with assurance as to its immediate practical application to everyday problems

*Enzymes**

THE authors have endeavored to collect in concise form all the available information in regard to enzymes because as they remark in their preface, anyone who attempts a study of enzymes cannot fail to be struck by the vast accumulation of literature on the subject, while at the same time he will be confused by the many apparently contradictory results which have been published

Modern studies of the chemistry of the living organisms both in health and disease, and the development of a broader and better understanding of the chemical activities of the bacterial causes of disease as well as what Wells has aptly termed the “chemical pathology” resulting have greatly extended this field of study

The authors have endeavored to coordinate and correlate all the essential studies on this subject and in so doing have presented a most excellent monograph which should prove invaluable to all workers alike

The clinician, the pathologist, and the laboratory worker alike will find this volume most useful, as—to quote again—enzymes are formed by all living cells, whether the latter carry on all the functions of an organism as in the case of unicellular forms of life or are devoted only to specialized functions as in higher plants and animals

“Life,” say the authors ‘is just one enzyme reaction after another,’ and indeed, to a large extent a somewhat similar phrase could be applied to disease

This monograph is not only exhaustive but excellently arranged and written

It is divided into four major sections I, Properties of Enzymes, 57 pages II Distribution of Enzymes 60 pages III Methods for the Preparation and Study of Enzymes, 97 pages IV Practical Applications of Enzymes 92 pages

Methods are well described generally those which the authors have found satisfactory in their own experience A bibliography of 1323 references is appended evidencing the thoroughness with which the subject is reviewed

The craftsmanship of the publishers is excellent though one might wish for a smoother finish paper

This work may be heartily recommended

The Aspergilli†

THE authors remark very pertinently in the introduction to this book that the aspergilli—the “weeds” of the culture room—form a very considerable percentage of all the mold colonies encountered in the examination of soil foodstuffs, and miscellaneous material

In spite of their frequency these forms have been neglected and the literature concerned with their occurrence characteristics, and relation to human and animal life is in a very chaotic and confused state

The authors have been engaged in a study of this genus since 1904 and the present

Enzymes By S. A. Waksman Associate Professor of Soil Microbiology Rutgers University and W. C. Davison Associate Professor of Pediatrics Johns Hopkins University Pp 364 Cloth. Price \$5.50 net. Williams and Wilkins Co Baltimore

†*The Aspergilli*. By Charles Thom and Margaret B. Church of the Microbiological Laboratory of The Bureau of Chemistry Department of Agriculture. Pp 74 plates and 13 figures Cloth. Price \$5.00 net. Williams and Wilkins Co Baltimore

volume, embodying the results of long study of about 350 strains, is frankly biologic and primarily taxonomic in purpose

It reflects in every page the extensive experience and the arduous labor of its authors and furnishes for the first time within the covers of one book a succinct but comprehensive discussion of the aspergilli which should prove of incalculable value

After an historic discussion, followed by the description of a generic characterization, the morphologic classification is clearly discussed Chapter III gives the basis of description and classification Chapter IV is devoted to cultural methods

In the following chapters are discussed the physiologic and biochemic activities of aspergilli, the industrial significance of their enzymic and fermentative activities, and their relation to animal diseases

The remaining 158 pages are devoted to group keys and a comprehensive description of those species which have been definitely classified The final portion of the book presents a synoptic key, a list of accepted species and a list of 127 references

The book thus presents, for the aspergilli, a source of reference similar to those available for the classification and identification of bacteria and should be at hand in every laboratory concerned with botanical or bacteriologic studies or food investigations

The book is well bound and printed

*Hydrogen-Ion Concentration**

THIS book, for the sake of continuity with its smaller predecessor, is called a second edition, but in view of the extensive as well as comprehensive expansion that this volume has undergone, it may well be regarded as a new book

Neither Professor Michaelis nor his qualifications as an authority in this field require extended discussion

So vast has been the amount of work done with regard to the significance of hydrogen ion concentration in connection with biological sciences and so rapidly have technical procedures concerned with its determination undergone improvement that, in order to cover the field completely, a series of volumes is in contemplation, this being the first and concerned only with the theoretic physicochemical principles involved

In this volume, because of the broadened realm of pure physical chemistry and the extraordinary growth of the multiplicity of applications of this branch to the other branches of science, the fundamental principles are placed upon a wider basis than before

The volume deserves a wide circulation and should prove a valuable source of reference

*Hydrogen-Ion Concentration By L. Michaelis, Lecturer in Research Medicine Johns Hopkins Hospital translated into English by W. A. Perle, Associate in Medicine Johns Hopkins Hospital Second Edition Pp 299 32 figures Cloth Price \$5.00 net. Williams & Wilkins Co., Baltimore

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO, FEBRUARY 1927

No 5

Editor in Chief WARREN T VAUGHAN, M D
Richmond, Va

ASSOCIATE EDITORS

DENNIS E JACKSON M D	CINCINNATI
PAUL G WOOLLEY M D	LOS ANGELES
J J R MACLEOD M B	TORONTO
W C MACCARTY M D	ROCHESTER, MINN
GERALD B WEBB M D	COLORADO SPRINGS
VICTOR C MYERS PH D	IOWA CITY
RUSSELL L HADEN M D	ROSEDALE ILL
JOHN A. KOLMER M D	PHILADELPHIA
ROBERT A. KILDUFFE, M D	ATLANTIC CITY, N J
A. PARKER HITCHENS M D	MANILA P I
DEAN LEWIS, M D	BALTIMORE
GEORGE R. HERRMANN M D	NEW ORLEANS
HAROLD D CAYLOR M D	ROCHESTER MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927, by The C V Mosby Company--All Rights Reserved
Entered at the Post Office at St Louis Mo as Second Class Matter

EDITORIALS

Liver Function Test

IN RECENT years much attention has been devoted to studies of liver function and to the development of clinically applicable means for measuring its efficiency or degree of impairment.

Probably no exaggeration is involved in considering the liver as one of the most important organs of the body, certainly there are few whose functions are more multiple.

Carlson¹ renewing our present knowledge of hepatic function cites the following

1 Fairly conclusive experimental evidence is available that the liver is concerned with the coagulation of the blood in that fibrinogen is either produced in the liver or under its influence certainly at all events this is true of the regeneration of fibrinogen after hemorrhage.

It is also known that under certain circumstances the liver may produce substances capable of both acceleration and retardation of coagulation and

evidence exists indicative of the relation of the liver to various hemorrhagic diseases, particularly in infancy

2 It is probable, though not as yet definitely proved, that the liver is the chief organ producing urea from the cleavage products of protein metabolism and from ammonia absorbed from the alimentary tract

3 The intimate relation of the liver to carbohydrate metabolism, and its importance in relation to hypoglycemia, hyperglycemia, diabetes, and various forms of glycosuria is a problem not yet fully worked out but the importance of which is fully recognized and upon the study of the intricate mechanism of which a vast amount of work has been and is being done

4 That there is some relation, also, between the liver and fat metabolism is entirely probable, related, very possibly, to fat desaturation and oxidation

5 As a source of bile, which seems both an excretion and secretion, the liver is of primary importance and the center of an intricate and far reaching mechanism

6 There is, finally, an interlocking relation between the nervous system and bile evacuation as well as liver function in general which, as Carlson remarks, forms an interesting and challenging chapter in the literature of liver function in health and disease

In spite of the importance given to the liver even from the days of Hippocrates and the fact that it has long been regarded as exerting marked influence upon the functions of the body at large, its multitudinous functions are, apparently, only gradually coming to light

Mann² has shown, in corroboration of others, that, following hepatectomy in dogs, marked changes occur in carbohydrate and protein metabolism and in the constituents of the bile with resultant disturbance of the bodily functions in general

Health, in general, may be regarded as a condition characterized by, if not dependent upon, perfect performance of function. Disease, in turn may be broadly described as characterized or evidenced by disturbance of function. The essential value, therefore, of the development of means for the detection of functional inefficiency or the measurement of the degree of impairment is obvious and much attention has been devoted to the evolution of liver functional tests

Because of the diversified nature of hepatic functions the problem is by no means an easy one and many methods have been proposed

Rowntree, Marshall, and Chesney³ have listed among the methods proposed

1 Carbohydrate tests inconstant and unreliable

2 Nitrogenous studies of the urine unreliable

3 Tests for urobilin useful as signifying liver damage but futile as a measure of the extent or kind of damage

4 Determination of fibrinogen content of the blood because of the importance of the liver as a source of fibrinogen

5 Estimation of blood lipose, because this is increased after liver damage experimentally produced

6 The phenoltetrachlorophthalein test

7 Tests for fibrinolytic ferment in the blood

The two tests at present attracting particular attention are based upon (a) the ability of the liver to remove certain substances from the blood with such accuracy that their impaired elimination may serve as a measure of hepatic injury, and (b) studies focused upon the most obvious hepatic secretion—and excretion—the bile

In the first group the most work has been done with phenoltetrachlorophthalein, a dye substance first introduced by Rowntree Huitwitz and Bloomfield⁴ which is specifically excreted by the liver and which Whipple, Peigenthal, and Clark showed might be used as an index of liver damage

Liver function may be measured to some extent, by the intravenous injection of a known amount of this dye and the subsequent determination of the degree to which it has been removed from the blood after a definite interval

Numerous reports have been made upon this procedure

Friedenwald and Gault⁵ using the duodenal tube method in 169 tests on 69 cases, report that there were but slight daily variations in the rate of excretion of the dye in the bile in the same individual that starvation, age or sex were without effect and that the normal average appearance time of the dye in the bile was 13.8 minutes

A slight prolongation was noted in pregnancy (8 cases) diabetes (2 cases) exerted no effect in epilepsy (3 cases) a slight prolongation occurred (14.9 minutes) and also in malnutrition (2 cases) 15 minutes, while in cirrhotic jaundice (2 cases) the excretion time was 10.5 minutes

The following findings were encountered in the conditions listed Addison's disease (1) 14 minutes hyperpituitarism with epilepsy (1) 24 minutes, thyrotoxicosis (1), 17 minutes secondary syphilis (2) 16 minutes, typhoid fever (2) 15.8 minutes gallstone (40) 17 minutes stone with jaundice (9) 28 minutes and (5) none in 60 minutes hepatic carcinoma (1) 25 and 45 minutes, atrophic cirrhosis (2) 45 minutes and none in 60 minutes arsenphenamine jaundice (1), none in 2 hours cancer of the pancreas (1), none in 2 hours, cardiac disease (1) myocarditis 18 minutes (1) chronic passive congestion, 13 minutes

Ottenberg, Rosen and Goldsmith⁷ using the Rosenthal technique and determining the amount of dye remaining in the circulation after 15 and 60 minutes in 100 cases (in 14 cases after 1, 2 3 5 and 10 minutes) found it to be very rapidly removed probably because 75 per cent of the total blood volume passes through the liver within two minutes and 96 per cent within five minutes

They contend that this removal of the dye from the blood takes place whether the liver is able to excrete the dye or not and therefore that it is dependent not upon excretion by the liver but upon absorption from the blood The amount of dye in the circulation at the end of 15 minutes indicates according to these observers the equilibrium between the presence of the dye in the blood and the absorptive capacity of the tissues this point of

diac cases the index varied with the degree of compensation, cases with high readings proving fatal

Similar reports are made by Maue¹ and many others

There are numerous reports of investigations of Van den Bergh's test substantiating its clinical applicability so that it may be said with confidence that the methods concerned with the detection and measurement of bile pigments in the blood furnishes, at present, the method of choice in studies of hepatic functional impairment

REFERENCES

- ¹Carlson, A. J. Physiology of the Liver Present Status of Our Knowledge, Jour Am Med Assn, Nov 7, 1925, lxxxv, 1468
- ²Mann, F. C. Modified Physiologic Processes Following Total Removal of the Liver, Jour Am Med Assn, Nov 7, 1925, lxxxv, 1472
- ³Rowntree, L. G., Marshall, E. K., and Chesnev, A. M. Studies in Liver Function, Tr Assn Am Phys, 1914
- ⁴Rowntree, L. G., Hurwitz, S. H., and Bloomfield, A. L. Bull Johns Hopkins Hosp, 1913, xxiv, 327
- ⁵Whipple, G. H., Peighthal, T. C., and Clark, A. H. Bull Johns Hopkins Hosp, 1913, xxiv, 343 Cited by Opie, E. L. Jour Am Med Assn, Nov 14, 1925, lxxxv, 1533
- ⁶Friedenwald, J. and Gautt, W. H. Observations on the Phenoltetrachlorphthalein Test as a Means of Determining Liver Function, Am Jour Med Sc, October, 1923, clxvi, No 4, p 619
- ⁷Ottenberg, R., Rosen, S., and Goldsmith, L. The Removal of Phenoltetrachlorphthalein from the Circulation, JOUR LAB AND CLIN MED, November, 1924, 2, No 2, p 138
- ⁸Piersol, G. M., and Bockus, H. L. Comparative Studies in Liver Function by Some of the Later Methods, Jour Am Med Assn, Oct 4, 1924, lxxxiii, 1044
- ⁹Hovic, G. H. Phenoltetrachlorphthalein Liver Function Test, Jour Am Med Assn, Feb 2, 1924, lxxxiii, 361
- ¹⁰Boardman, W. W. and Schoonaker, G. D. Phenoltetrachlorphthalein in the Study of Liver Function, Am Jour Med Sc, November, 1924, clxviii, No 5, p 688
- ¹¹Greeubbaum, S. and Brown, H. Phenoltetrachlorphthalein Liver Test in Acute and Chronic Syphilis under Treatment and in Various Skin Diseases, Jour Am Med Assn, Jan 12, 1924, lxxxiii, 88
- ¹²Rosenfeld, H. H. and Schneider, E. F. Improved Phenoltetrachlorphthalein Test for Liver Function in Pregnancy, Jour Am Med Assn, March 17, 1921, lxxx, 743
- ¹³Bogen, E. A. Clinical Test for Liver Function, JOUR LAB AND CLIN MED, June 1923, viii, 9
- ¹⁴Maurer, S. and Gatewood, L. C. Phenoltetrachlorphthalein Liver Function Test, Jour Am Med Assn, March 28, 1925, lxxxiv, 935
- ¹⁵Kerr, J. W., Delpratt, G. D., Epstein, N. M., and Dunievitz, M. Rose-Bengal Test for Liver Function, Jour Am Med Assn, Sept 26, 1925, lxxxv, 942
- ¹⁶Tada, Y. and Nakashima, K. A New Dye for Test of Liver and Biliary Tract Function, Jour Am Med Assn, October 25, 1925, lxxxviii, 1292
- ¹⁷Rosenthal, S. and White, E. C. Clinical Application of the Bromsulphalein Test for Hepatic Function, Jour Am Med Assn, April 11, 1925, lxxxiv, 1112
- ¹⁸Muehlingracht. Deutsch Arch f klin Med, 1921, cxxvii, 1
- ¹⁹Muller, G. P., Ravdin, I. S., and Ravdin, E. G. Alterations of Bile Pigment Metabolism in Biliary Tract Disease, Jour Am Med Assn, July 11, 1925, lxxxv, 86
- ²⁰Bernheim, A. Icterus Index, Jour Am Med Assn, Jan 20, 1924, lxxxiii, 291
- ²¹Maue, H. P. Icterus Index of the Blood Serum, Surg Gynec and Obst, June, 1922, xxxiv, 752

—R A K

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., Editor in Chief
Medical Arts Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.B.
University of Toronto Toronto

Pathology

PAUL G. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD E. WEBB, M.D.
Cragmor Sanatorium Colorado Springs

Clinical Pathology

W. C. MACCARTY, M.D.
Mayo Clinic Rochester Minn.

HAROLD D. CATLOR, M.D.
Mayo Clinic Rochester Minn.

Biochemistry

VICTOR C. MYERS, Ph.D.
State University of Iowa Iowa City

Experimental Medicine

RUSSELL L. HADEN, M.D.
University of Kansas Rosedale

Immunology

JOHN L. HOLMER, M.D.
University of Pennsylvania
Philadelphia

ROBERT A. AILDUFFE, M.D.
Atlantic City N. J.

Bacteriology

A. PARITER HITCHENS, M.D.
Bureau of Science, Manila, P. I.

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY 323 25 First Blvd. St. Louis
Entered at the Post Office at St. Louis Mo. as Second Class Matter

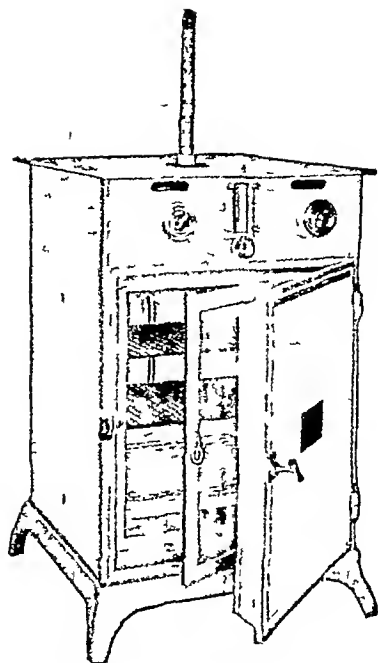
LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment. Estimates Cheerfully Furnished Prompt Service.

See our advertisements pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA, PA.



Freas Incubator No 26

Your Incubator Must be Accurate

Just as with your laboratory worker your incubator must be capable of performing the assigned tasks accurately

Accuracy in an incubator is its ability to maintain the temperature constant continuously

This is why the Freas Incubators are used in the leading laboratories

The Thermo Electric Instrument Co.
1215 South Grove Street, Irvington, N. J.

FREAS

ELECTRIC INCUBATORS

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO MARCH 1927

No 6

CLINICAL AND EXPERIMENTAL

THE VAN DEN BERGH REACTION FOR SERUM BILIRUBIN WITH NOTES ON INTERPRETATION AND TECHNIC*

BY W W HALL M D WASHINGTON, D C

LIVER function tests are of late coming rapidly to the fore. The work done by the physiologist in establishing methods for the study and evaluation of the normal and the work of the clinical pathologist have combined to give us a number of tests by means of which the various functions of the liver may be studied.

Some of the proposed procedures seem to have very little foundation when studied by experimental physiology.¹ This may be because disease affects the liver function in a different way than simple removal of increasing amounts of hepatic tissue, disease may cause a qualitative as well as a quantitative deviation from the normal. The physiology of the human liver may differ from that of the experimental animal or because the test used is an "index of disease not necessarily wholly hepatic."²

The van den Bergh appears to be one of the liver function tests for which the least that may be said is that it serves to measure degree and differentiate type of bilirubinemia in the causation of which the liver may be directly or indirectly but not necessarily alone at fault. Van den Bergh developed his reaction by applying the Ehrlich diazo reaction to sera containing bilirubin. The reaction depends upon the formation of azobilirubin, a red dye, when an acid solution of a diazonium salt is added to bilirubin in solution. Van den Bergh found that pure bilirubin in a dilution of 0.7 mg per liter gave a positive reaction and that allied substances such as biliverdin did not.

Two types of jaundice are differentiated by the van den Bergh reaction and their differentiation depends upon the fact that bilirubin in one type (obstructive) combines promptly with the diazo reagent to form azobili-

From the Laboratories of the U S Naval Medical School Washington D C
Received for publication November 18 1926

rubin (direct reaction), while in the other (hemolytic) type it seems to be bound in such a way that the reaction is long delayed or negative, but if alcohol be added the reaction occurs promptly (indirect reaction). The two general groups of jaundice, obstructive and hemolytic, are caused by pathologic lesions of distinctly different type and location.

A résumé of the theory of bilirubin formation and excretion may be of help in the discussion of van den Bergh's classification. Erythrocytes are constantly being destroyed in the body and bilirubin is formed from the liberated hemoglobin by cells of the reticuloendothelial system. These cells are found throughout the body in the endothelium of vessels and capillaries but most abundantly in the sinusoids of the spleen, lymph glands and liver.

The bilirubin thus formed is presumed to be present in combination in the blood stream. As the blood passes through the liver the parenchymatous or polygonal cells extract the bilirubin and excrete it into the bile canaliculi. When for any reason the flow of bile is obstructed this pigment (with the bile salts) passes again into the blood by absorption. In this type of jaundice the bilirubin combines directly with the diazo reagent, upon its addition, to form the red azo dye. That pigment, present in small but constant quantity normally and in much larger amounts in conditions characterized by increased destruction of red cells, reacts only in the presence of alcohol which is thought to split it from its protein complex. Van den Bergh calls the former type of pigment obstructive and the jaundice in those conditions mechanical, while the jaundice of hemolysis he calls dynamic.

S. M. Rosenthal² says that in attempting to discover the mechanism by which certain dye stuffs and bilirubin are excreted by the liver he has studied their behavior from a physicochemical standpoint. By ultrafiltration experiments he has determined that they circulate firmly bound to the serum proteins. This prevents their elimination by the kidneys. Bile salts by their effect on surface tension are able to liberate bilirubin and these dye stuffs from their adsorption compound with the protein so that they can be further excreted by the liver.

Van den Bergh and his followers originally considered bilirubin in the two types of jaundice to be essentially different, the bilirubin which passed the polygonal cells of the liver having undergone some fundamental change as it thereafter reacted as the bilirubin in the bile itself. The essential difference is apparently not in the bilirubin itself but rather in the presence or absence of substances which act as do the bile salts to split the bilirubin from its protein complex and allow prompt union with the diazo reagent.

The rôle of the liver in bile pigment metabolism has recently been quite definitely established by Mann's³ experiments. He has proved that following total removal of the liver bilirubin rapidly accumulates in the blood, thus establishing the fact that the liver acts principally, with reference to bilirubin, as an excretory organ and that the transformation of hemoglobin to bilirubin is carried on efficiently quite independently of the parenchymal cells of that organ, presumably by the reticuloendothelial cells throughout the body.

McNee (as quoted by Bockus and Shay⁴) classifies jaundice as (1) obstructive hepatic, (2) toxic and infective, (3) hemolytic, and cites the biphasic

reaction (discussed under interpretation) as characteristic of the toxic and infective group. The reactions with blood from cases belonging clinically to this group have been very variable, some being prompt, direct, some delayed and others negative direct. Recent work indicates² that bile salts are synthesized as well as excreted solely by the liver and that the amount of bile salts formed may be diminished in some cases of liver malfunction. In view of the probable role of bile salts in the van den Bergh (qualitative) this may prove of importance in the interpretation of the reaction and help to explain some apparent contradictions in the toxic and infective group as well as with blood from patients in the early stages of obstructive jaundice.

Technic of the Method—Draw 5 cc of blood by venipuncture into a dry centrifuge tube and allow to clot. Separate serum by centrifuge if necessary, and pipette off. The diazo reagent which must be made up just before use is a mixture of two solutions:

<i>Solution A*</i>		
Sulphanilic acid	-	10 gm
Conc. HCl	- - -	150 cc
Distilled water qs	---	1000 cc
<i>Solution B</i>		
Sodium nitrite	-	0.5 gm
Distilled water	- - -	1000 cc
<i>To prepare fresh reagent</i>		
Solution A		2500 cc
Solution B	-	075 cc

*Reagents must be of best quality. Sulphanilic acid must be reasonably fresh. Very old samples have failed to react well.

*The Qualitative or Direct Reaction**—Place 0.25 cc serum in each of three small test tubes. To tube No. 1 add 0.2 cc water. To tube No. 3 add 0.2 cc diazo reagent (fresh). After waiting five minutes for reaction to become complete in control tube No. 3 add 0.2 cc diazo reagent to tube No. 2. Watch and time development of any reaction. Prompt or immediate reaction begins before thirty seconds have elapsed. Comparison with serum control tube No. 1, and completed reaction control, tube No. 3 will aid in detection of color.

The Quantitative Test or Indirect Reaction†—To 1 cc serum in a 15 cc graduated centrifuge tube add 0.5 cc diazo reagent (freshly prepared as above). After a minute or two add 2.5 cc 95 per cent alcohol and 1.0 cc saturated solution of ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$. Mix well with a stirring rod after each addition and finally centrifuge.

The diazo reagent is added before the alcohol to allow 'coupling' to take place. By this method very little if any bilirubin is carried down with the precipitated protein, as the azobilirubin is very soluble in alcohol while bilirubin is less so and is carried down with the precipitate in relatively large amounts if the reagents are added in the reverse order. The color of the supernatant fluid will vary from a faint pink color as in normal serum to a deep violet depending on the amount of bilirubin present. (Chylous sera give cloudy solutions which are objectionable for colorimetric comparisons.)

From note on improvement in technic. We have however discarded the use of caffeine sodium salicylate for we have found that its effect was very inconstant as did McNeely and Keefe.

†Quantitative method modified from Rivdin.

The quantity of supernatant fluid is read on the graduations of the centrifuge tube (Fig 1) and the dilution of the bilirubin contained in the cubic centimeter of serum used is thus directly obtained. The quantity of bilirubin present in the serum (1 cc) is now, as azobilirubin, entirely in alcoholic solution. This supernatant alcohol usually varies from 25 to 30 cc. The calculation of the dilution (1 in 4) as used by Ravdin⁷ and others does not appear to be accurate. We have had no difficulty in reading the amount of supernatant alcoholic solution, as the ammonium sulphate, protein and alcohol layers separate very sharply on centrifuging (Fig 1). As the color of the standard represents a bilirubin concentration of 5 mg per liter, the calculation is

$$\frac{\text{Standard}}{\text{Unknown}} \times \text{Dilution of Unknown} \times 5 = \text{mg bilirubin per liter of serum, (using a plunger type of colorimeter)}$$

$$\frac{\text{Unknown}}{\text{Standard}} \times \text{Dilution of Unknown} \times 5 = \text{mg bilirubin per liter of serum, (using a dilution type of colorimeter)}$$

Standard for the quantitative reaction *

Solution 1

Ammonium ferriolum -----	0.1508 gm	
Conc. HCl -----	50.0	cc
Distilled water qs -----	100.0	cc
(Keeps indefinitely)		

Solution 2

Of solution No. 1 -----	10.0	cc
Conc. HCl -----	25.0	cc
Distilled water qs -----	250.0	cc
(Keeps about one month)		

Standard which is made fresh daily

Of solution No. 2 -----	3.0	cc
10% ammonium sulphocyanate or		
20% potassium sulphocyanate -----	3.0	cc
Ether -----	12.0	cc

*As read from the supernatant alcoholic solution in graduated centrifuge tube

Shake thoroughly. The ether extracts the color from the solution and forms a supernatant layer which may be used in colorimetric comparison. The standard matches in color a dilution of 5 mg per liter of bilirubin.

By the use of cobaltous sulphate as suggested by van den Bergh and first published by McNee and Keefer⁸ a permanent aqueous standard may be made which avoids many of the errors and difficulties inherent in the ether standard. They advise the use of 2.161 gm anhydrous cobaltous sulphate to 100 cc water. This standard also represents the color given by 5 mg bilirubin per liter. We, however, found it difficult to obtain or make anhydrous (CoSO₄) cobalt sulphate as decomposition took place while the water of crystallization was being driven off. It is also impossible to obtain an accurate weight using the crystalline salt, allowing for the seven molecules of water of crystallization (CoSO₄·7H₂O), as the salt is somewhat efflorescent and perfect crystals almost never are found. We, therefore, suggest that the cobalt sulphate standard be made up as follows. Make an aqueous solution somewhat deeper in color than the ether standard, compare in colorimeter and dilute as indicated to match the color in the ether standard. This solution keeps well in the dark. We have found that the addition of 0.5 cc

H_2SO_4 , per 100 c.c. does not change the color and the solution keeps thus indefinitely

Interpretation—The normal range of bilirubin as given by McNee⁶ Raydin⁷ and others is from 1 to 3 mg. per liter, latent jaundice from 4 to 20 and clinical icterus from 20 up. The dividing line between latent and clinical icterus should probably be one or two points lower. We have seen some cases which were clinically mildly jaundiced with only 18 mg. bilirubin per liter serum. If reported in units one unit equals 5 mg. bilirubin per liter.

There are three possible results in the direct reaction

Immediate or prompt, beginning before thirty seconds have elapsed and reaching its maximum in about two minutes



Fig. 1—Quantitative van den Bergh reaction completed. Note sharply separated layers (1) Supernatant alcoholic layer containing in solution as azobilirubin all the bilirubin present in the serum added (1 c.c.) (2) Layer of precipitated proteins (3) Ammonium sulphate layer. The supernatant fluid varies in each test according to evaporation and fluid interchange but averages about 3 c.c. The dilution of bilirubin is therefore 1 in 3 (approximately). The exact amount must be read in each case and that figure used.

Delayed, beginning after thirty seconds. These reactions develop slowly. The longest in our experience has been thirty minutes although McNee and Keefer⁸ report delayed reactions which took one hour to develop.

Negative, no color developed in thirty minutes.

The prompt direct reaction is given by the bilirubin in the obstructive type of jaundice. Delayed or negative reactions may be obtained in both normal sera and those from cases of nonobstructive or hemolytic jaundice. We have dropped the term 'biphasic' reaction which was used to describe a reaction beginning promptly and not reaching its maximum until after thirty seconds, since we found as did Andrews⁹ that no specimens, no matter how intense a

prompt direct reaction they gave, developed their maximum color before one to two minutes had elapsed. We have therefore classed as prompt direct any reaction beginning before thirty seconds. The delayed and negative direct reactions may be grouped together as both may be obtained in normals and in nonobstructive jaundice.

The direct reaction serves both to measure the bilirubin and to develop a color with bilirubin in the presence of alcohol which gave none in the direct reaction. Thus it demonstrates and measures the jaundice of hemolytic origin and brings to light a jaundice of latent type, that is, one in which the concentration of bilirubin has not reached the level at which it can be demonstrated in the urine by the usual tests nor can be detected clinically in the sclera and skin. We have found no case in which color was entirely absent in the quantitative or indirect reaction, although many, in fact most, normals gave a reading of less than 1 mg per liter (0.2 unit). All types of bilirubin give color in the indirect reaction, so that to report a positive indirect reaction without reference being made to the type of direct reaction is to give incomplete information.

We do not agree with McNee and Keefer⁶ that the reactions change on standing and that specimens which originally gave a prompt direct reaction give a long delayed reaction later. We have kept sera as long as three months and at the end of that time they still gave a prompt direct reaction as before.⁹ Slight hemolysis has not interfered with the reactions.

SUMMARY AND CONCLUSIONS

1 The van den Bergh reaction is a means of qualitative (direct reaction) and quantitative (indirect reaction) study of serum bilirubin. The presence of bile salts in serum of obstructive jaundice probably explains the prompt direct reaction of the bilirubin in this type and is the essential difference in sera of obstructive and hemolytic jaundice.

2 The term biphasic was used to describe a direct reaction which began before thirty seconds had elapsed, following the addition of the diazo reagent, and increased in intensity for some time after the thirty second limit. This designation (biphasic) is misleading and should be dropped, for no sera, even those most heavily jaundiced (obstructive), reach their maximum color until long after thirty seconds have elapsed. We have therefore classed the direct reactions as follows: prompt or immediate, any reaction beginning in thirty seconds after addition of the reagent to the serum; delayed, a reaction beginning slowly after thirty seconds and reaching its maximum very slowly, sometimes taking thirty minutes or longer; negative, those in which no color at all developed.

3 In calculation of the dilution in the quantitative reaction the quantity of supernatant alcoholic solution should be read and that figure used (Fig 1), as the bilirubin in the serum (1 cc) is all present as azobilirubin in the alcohol.

4 The aqueous cobalt sulphate standard is more desirable than the ether standard. It is more easily handled, is not subject to rapid evaporation and

can be made up in quantity. Comparison with the ferric sulphocyanate ether standard is recommended as a means of avoiding gravimetric errors if a crystalline cobaltous sulphate is used ($\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$).

5 Addition of a small amount of concentrated sulphuric acid (0.5 cc to 100 cc of standard) does not change the color of the cobalt standard and helps preserve it.

6 Serum bilirubin reactions do not appear to change on standing if refrigerated.

REFERENCES

- ¹Mann, Frank C and Bollman, Jesse L. Liver Function Tests Arch Path and Lab Med 1926, 1, No 5 641
- ²Rosenthal, S M. Discussion on Liver Function Jour Am Med Assn Nov 14 1925 LXIV, 1549
- ³Mann, Frank C. Modified Physiologic Processes Following Total Removal of the Liver Jour Am Med Assn Nov 7 1925 LXIV, 142
- ⁴Bockus, Henry Leroy and Sherr, Harry. Obstructive Jaundice Med Clin No America, September 1926 p 379
- ⁵Smyth, F S and Whipple G H. Bile Salt Metabolism I. Influence of Chloroform and Phosphorus on Bile Fistula Dogs Jour Biol Chem April 1924 LX 63. Whipple G H. Origin and Significance of Constituents of Bile Physiologic Review July 1922, 11 440
- ⁶McNee J W and Keefe, Chester S. The Clinical Value of the van den Bergh Reaction for Bilirubin in the Blood Brit Med Jour July 11 1926 No 3367 p 52
- ⁷Rardin, Elizabeth Glenn. An Estimation of the Clinical Value of the van den Bergh Test Am Jour Med Sci clix p 800
- ⁸Andrews. Lancet, London 1924 1 510 (as quoted by McNee)⁶
- ⁹Hall W W. Useful Hepatic Function Tests Naval Medical Bulletin October 1926 xxiv 4 p 843

NOTES ON SICKLE CELL ANEMIA*

By GEORGE S GRAHAM, M D , AND SARAH H McCARTY, A B , BIRMINGHAM, ALA

IN 1910 Henrick described a case of severe anemia in which the red cells were elongated and sickle shaped¹ During the following thirteen years, three similar cases were recorded by Washburn,² Cook and Meyer,³ and Mason⁴ In all of these cases the arresting sign had been a peculiar deformity of the red cells as observed in the blood film The clinical findings associated with the erythrocytic anomaly seemed to outline so definite a syndrome that Mason wrote of his case as an example of "Sickle Cell Anemia"[†] While studying the case of Cook and Meyer, Emmel⁷ found that the cell deformity was greatly accentuated when a fresh blood drop was sealed beneath a cover slip and examined after a lapse of some hours Not only did the deformity increase but the percentage of affected cells mounted rapidly In fresh preparations from the case studied about one-third of the cells were elongated, curved or crescentic In sealed preparations the deformity was greatly exaggerated and the number of affected cells increased until in twenty-four hours practically 100 per cent of the red cells had become converted into various bizarre shapes (Fig 1)

The first real understanding of this curious condition dates from 1923 when two illuminating papers were published, one by Sydenstricker, Mulheim and Houseal,⁸ the other by Huck⁹ Following out certain leads from earlier work and adding further findings of their own, they demonstrated that an anemic syndrome characterized by sickling deformity of the red cells is relatively common and is not, as had been supposed, a medical oddity They verified its striking familial character and both were able to collect cases readily by examining family groups introduced into attention by the discovery of an affected member These papers focused attention sharply upon the condition and it is now widely recognized, at least among physicians of the South But its real frequency is not yet fully appreciated

Thus far the red cell anomaly has been demonstrated only in the full or part-blood negro Sydenstricker¹⁰ is quoted as having examined 1000 white patients without finding a single instance We have made no systematic attempt to follow this line of inquiry We have set up occasional preparations from cases of various blood diseases, however, with negative

*Read at the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas Texas April 1926

†In 1904 Dresbach⁵ reported the occurrence of elliptical red cells in the blood of a mulatto medical student No other abnormalities were found in the blood and there was no clinical evidence of anemia Dr Dresbach has kindly furnished us a photo of the stained blood film from his case He states that cover slip preparations were made of the blood for purposes of class demonstration and that some of these were examined at intervals probably as long as fifteen hours after mounting but that no further evidences of form change were seen Bishop recorded a similar erythrocytic deformity in 1914⁶ The patient's race was not stated The subsequent history of Dresbach's patient is suggestive of that common in sickle cell anemia but it is doubtful whether either of these cases was an instance of the disease

results. Should continued study fail to show the occurrence of the anomaly in the white race there still remain interesting questions as to its possible occurrence in other races and as to its rate of incidence and its manifestations in the black populations of other parts of the world. Information along these lines would be of considerable interest.

The clinical aspect has been so well covered by Sydeustrieker and by Huck that there is no occasion for detailed statement here. Common signs and symptoms are anemia with its concomitant malaise and disability, general bodily underdevelopment perhaps accompanied by a subnormal mentality, epigastric pain and low grade gastrointestinal disturbance, muscular or arthritic pain of variable location, greenish coloration of the sclera. General lymphadenoid enlargement occurs sometimes and there may be slight enlargement of the liver. The urine has a low fixed specific gravity. Albuminuria is usually found and there may be cylindruria. Urobilin is usually present. Bilirubinemia is very common in well developed cases and there

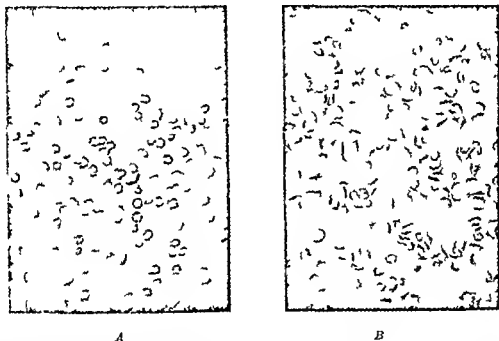


Fig 1 1.—Blood from a case of sickle cell anemia as observed immediately after it had been sealed beneath a cover slip.

B.—A similar preparation as it appeared twenty-four hours later. Red cell deformity or menisocytosis is now well developed.

may be jaundice. Chronic ulcer of the legs or ankles may occur particularly after childhood.

The sickling anomaly may be found in individuals who declare themselves well and strong and who give no history of any illness attributable to the blood dyscrasia. Sydenstricker regards such instances as examples of a 'latent phase' of the disease. In the majority of cases the affected individual presents some at least of the stigmata above outlined while in occasional individuals the condition becomes of major importance. There is now well marked anemia. The patient is puny, listless and easily fatigued. At intervals of weeks, months or years there are attacks of acute illness with exacerbation of the anemia and its attendant conditions. There are many evidences of active blood destruction. It is of particular interest that such attacks in the susceptible person may follow exposure to cold or damp

ness They may perhaps be brought on by overexertion In our experience these attacks are associated with evidence of some infection, particularly of the respiratory tract Huck was impressed with the susceptibility to pneumonia and tonsillitis, an opinion with which we agree In symptomless cases the sickling deformity may be the only definite abnormality of the blood There is, however, apt to be some anisocytosis and polychromasia Such abnormalities may become marked and may be associated with poikilocytosis and the appearance of erythroblasts even in individuals who make little complaint of ill health The white count is usually elevated, markedly so in active cases and here a few myelocytes appear Moderate eosinophilia is common There is a high percentage of reticulocytes during exacerbations, together with large numbers of nucleated red cells In an active case we once encountered 762 nucleated red cells in differentiating 500 leucocytes, while reticulocytes ran as high as 30 per cent Phagocytosis of the red cells by circulating endothelial leucocytes is a common phenomenon It may occur even in the absence of complaint and in patients who have good red cell counts and hemoglobin values

Even as brief an account as the above may serve to indicate how serious a problem a condition of this sort may become, particularly when it is remembered that the affection is not at all uncommon These individuals bear the constant burden of a blood dyscrasia whose outstanding feature is an abnormally high destruction rate of the erythrocytes With the advent of periods of stress such as may accompany infection or other untoward circumstance, they are correspondingly handicapped In medical, surgical, and obstetrical wards these are the patients who stay longest in the hospital and are most liable to complications and sequelae They appear to be relatively short-lived Among fifty-eight cases that have come under our observation, only two have been over fifty years of age One of these was fifty-six, the other fifty-seven, and both were of the inactive type Five of the series were newborn infants, four were children in the first decade of life, twelve were from eleven to twenty years of age, twenty-nine from twenty-one to thirty years of age, three from thirty-one to forty, three from forty-one to fifty The marked preponderance of patients in the second and third decades may be explained in part, though not completely, by the greater relative number of such individuals making up the general hospital population We do not yet know whether the condition may be recovered from in later life So far as present knowledge goes, this seems very improbable and we are forced to assume with Huck that most of these people die before they have passed the thirtieth year

We have been surprised at the frequency with which the sickling deformity can be demonstrated At different periods, each of several weeks' duration, we have set up routine cover slip preparations on all negro patients on whom blood counts were being made Medical, surgical and obstetrical patients were included We have thus examined the blood of 608 individuals Among them we have encountered forty-four instances of the sickling deformity For this series, then, the anomaly is demonstrable in 7.2 per cent of the patients in the negro wards of a county hospital In an earlier summary drawn

from 250 patients we found 52 per cent of 'sicklels'. We have attempted to follow family groups in only a few instances but among eleven immediate relatives of known sicklers eight were positive. Of six infants born of sickling mothers, five have been positive. Sydenstricker¹⁰ reports an incidence of $\frac{1}{4}$ of 1 per cent among the negroes examined at his clinic. His series is much larger than our own and it included a large number of out-patients that is of individuals probably suffering from minor complaints, but the disparity in the incidence rate is still striking. It is possible that our higher rate is due to our routine use of thin cover slips in the setting up of preparations. Certain puzzling experiences of our earlier work lead us to look for a purely physical explanation of the cell deformity and after various attempts at altering the physical and chemical conditions to which the cells were exposed *in vitro* we found among other things that a difference in the thickness of the cover slip under which they were compressed might promote

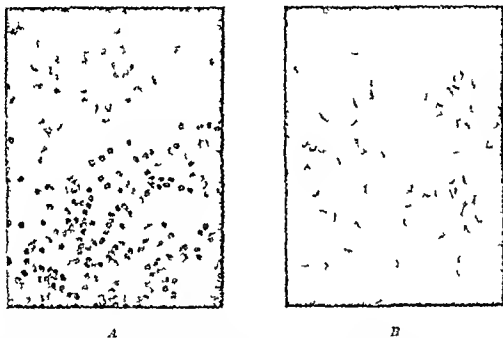


Fig. —Two cover slip preparations were made simultaneously for the same patient and upon the same slide. A was covered with a No. 0 cover slip. B with a No. 2 cover slip. Photographed twenty-four hours after set up.

or hinder deformity. A thin cover may elicit sickling while a thick one fails to do so. We have repeatedly found that when similar drops of blood are placed at opposite ends of a slide and covered one with a No. 0 $\frac{1}{8}$ inch cover square the other with a No. 2 square of the same size sickling appears promptly under the thin cover but is absent under the thicker (fig. 2). The difference is not so marked when No. 0 and No. 1 covers are used but it is still present in an occasional blood. In searching for the anomaly we have always used the No. 0 cover slip and have thus recorded occasional positive findings where thicker covers would have given negatives. In many of our cases as a matter of fact both thick and thin covers have been used.

In one case where sickling was active peculiar form changes were observed by watching the cells during the critical period of break up. While the red cell of normal blood appears homogeneous and firm it seemed here soft and unstable. Its substance appeared agitated by a play of forces that kept the surface membrane and contents in a continued state of instability.

We have had opportunity for making postmortem examination in four cases. A study of the first of these has already been reported.¹¹ None of the subsequent cases have been so well developed but each has been valuable as affording means for comparative study. A detailed histologic study of these cases will be reported upon at a future date but we wish to call attention at this time to certain findings that appear to us to have a possible bearing upon the general understanding of the condition.

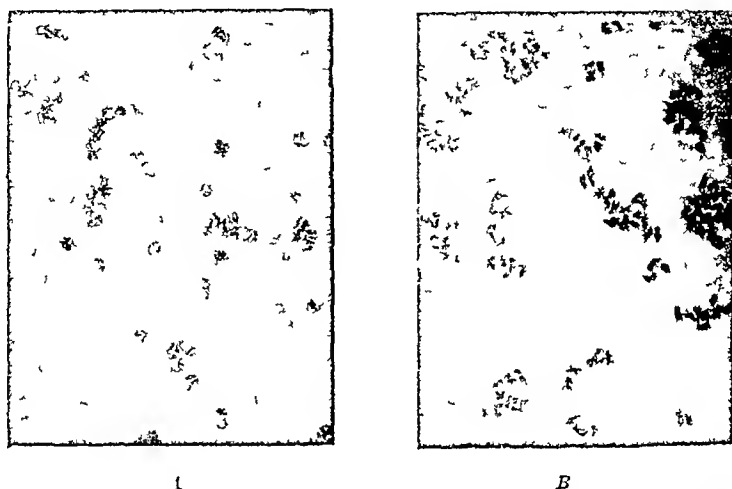


Fig 3—Cover slip preparation of blood taken from a vein during autopsy. "A" was photographed one hour after set-up. "B" about twenty-four hours later.

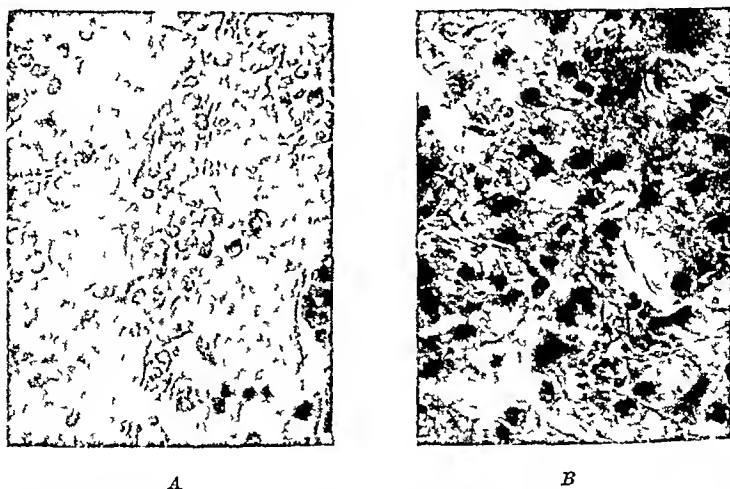


Fig 4—Sections from liver (Case II). "A" is from a block fixed in Zenker's solution. "B" from a block fixed in formalin. Both blocks were imbedded in paraffin the former by the chloroform method the latter by the simpler benzol method.

Of the four cases in the series, the first two had a similar clinical course. In Case I, a male, aged thirty years, death was due to streptococcal bronchopneumonia with associated bacteremia. Case II was that of a male, aged nineteen years. Death was again due to acute bronchopneumonia. In this case, as in the first, the lungs showed many small abscesses developing in

areas of peripheral infarction. *Streptococci* were present in these lesions, as well as in the pleural cavity. The autopsy was performed twenty seven hours after death. Blood taken from a vein during autopsy and sealed beneath a cover slip in the usual manner showed at first only occasional deformed red cells but next day there was typical sickling deformity in about 75 per cent of the cells (Fig 3). The same observation had been made in the first case. As in the first case also, it was found that paraffin sections of tissue fixed in Zenker's solution contained red cells of normal appearance, whereas parallel blocks from formalin fixed tissue showed well marked sickling deformity or at least an equivalent distortion of the cells (Fig 4). Case III was that of a truck driver aged twenty nine years who died of meningeal hemorrhage three days after the receipt of accidental cranial injury. The sickling deformity of his red cells was discovered only as the result of the routine examination that was being practiced on unselected patients. He had a red count of 4,360,000 and a hemoglobin of 90 per cent the highest values for any patient in our series. In Case IV the age was twenty three years. The patient had a positive Wassermann and cutaneous signs of a secondary luetic eruption. He died of peritoneal and generalized tuberculosis after an illness of about three months duration.

The one finding common to all these cases was as might be expected the evidence of a continued erythrocytic destruction rate higher than the normal. Such evidence consisted first in the widespread deposit of hemosiderin in the tissues, most marked in the spleen but present also in liver, bone marrow and lymph nodes. There was also definite hyperplasia of the bone marrow in the first two cases. The marrow was not obtained in Case III. In Case IV, the marrow of the femur showed well marked serous atrophy. In all four cases many phagocytic cells containing red cells or blood pigment were found in the great reticuloendothelial centers and such cells occurred also in occasional vessel lumina of various organs.

DISCUSSION

Following present usage, we have labeled as 'sickle cell anemia' every case in which sickling deformity has appeared in the red cells. But as knowledge of the condition increases it will probably become advisable to restrict the use of this term. In some of our cases there has been no clinically recognizable evidence of anemia at the time of examination. We believe that in the present state of our knowledge it would mile for clarity were we to look upon this cell deformity merely as one demonstrable stigma of an inheritable blood dyscrasia or status upon the basis of which there may, under conditions not yet understood, be built an anemic syndrome of well characterized type. If the problem be thus conceived, there is need for some word that may serve to designate the presence in a given blood of the sickling anomaly without at the same time committing us to any decision on the further question as to what other morbid changes may have made their appearance in the given case. To fill such need we have devised and would offer for adoption the term "meniscocytosis." It is derived from the Greek 'meniskhos' meaning "a sickle." As used, it would designate a peculiar and apparently

Areas of condensation or folds developed slowly or suddenly at the periphery and zones of rarefaction appeared alternately with fields of condensation in the cytoplasm so that the cell contents appeared to separate into globules or segments. Indentations or deep clefts developing at the surface were filled by a sudden rush of cytoplasm. The patterns produced by this slow kneading of membrane and contents were kaleidoscopic. A favorite formation was the development of equatorial or paracentric fissures one or more in number, often set at right angles to each other or arranged as multiple radiations from the middle region so as to set off several unequal sectors. The constantly changing contour was apt finally to develop a unilateral condensation or crescent and finally the tension set up somewhere at the surface appeared to become too great to be sustained and with a sudden snap the cell opened out into the characteristic "sickle" or changed more gradually into some irregular burred form while the cytoplasm flowed out at the ends or from multiple points about the periphery into thorny prominences or long, delicate streamers. The cell membrane or surface layer might appear to be retained, although it became wrinkled or stretched out into unusual shapes like an old piece of rubber that had lost its elasticity. But in some cases at least, the collapsed shell seemed to be partially or completely thrown off and the inner substance spread itself out irregularly.

We have not observed sickling in red cells that had been received into large volumes of various isotonic salt solutions, buffered or unbuffered, and set up in high dilution in such fluids. After centrifugation, however, sickling has been seen when the packed cells have been set up in these fluids without dilution. Here it is noticeable that the deformity begins about the periphery of platelet and leucocyte clumps, spreading slowly from such centers to involve wider fields. Washed cells sickle slowly and uncertainly in serum, but more normally in plasma. Defibrinated or oxalated blood also sickles slowly or imperfectly and again the change begins about platelet-leucocyte clumps. We have reached the tentative conclusion that the red cell deformity is determined *in vitro* by optimum pressure conditions plus biochemical activity of serum or plasma constituents. There is a strong suggestion that it is in some way related to the development of the fibrin net.

Evidence of a peculiar red cell structure is furnished by the findings of the "fragility" test. In several respects there are resemblances between sickle cell anemia and hemolytic jaundice. Here, however, there is sharp contrast. The red cells are found to have an increased rather than a diminished resistance to the hemolytic action of hypotonic salt solutions. Determinations made on twenty-four different individuals, some symptomless, others presenting various degrees of anemia and disability, have shown consistently low values for the point of complete hemolysis. This increased resistance is, in our experience, one of the most constant phenomena encountered, whether in active or in latent cases. Hemolysis begins at about the normal level. The highest value found was 0.48 per cent, the lowest, 0.325 per cent, with an average for all cases of 0.395 per cent. The highest level at which hemolysis was complete was 0.28 per cent, the lowest, 0.12 per cent, the average for the whole series being 0.19 per cent. In three cases the point of complete

hemolysis appeared to run lower than the levels quoted but in the absence of confirmatory readings these have not been included in the above figures

The sedimentation rate of the red cells is undoubtedly increased. We have made a few rough determinations on oxalated blood that had been collected as for routine blood chemistry. We used the simple expedient of drawing 1 cc of this blood into a 1 cc serologic pipette and measuring the amount of fall in the red cell column at ten minute intervals. The distance between the original level and the top of the descending red cell column was converted into percentage of the original total height. When thus set up along side normal controls, the settling rate of affected bloods is strikingly increased. Actual figures for one set up showed for the normal control a percentage decline of the red cell column during successive ten minute intervals of the first hour of 0.4, 1.3, 3.7, 6.9, 9.3 and 10.2. The blood of two obstetric patients taken during the puerperium showed 10, 5.3, 15.0, 22.3, 32.5, 37.5 in one case and 6.3, 11.3, 21.9, 31.3, 34.5, 38.7 in the other. That of a third (medical) patient showed 9.0, 22.5, 30.0, 35.0, 57.5, 60.0. This blood showed no further fall at eighty minutes and at two hours. The normal stood at 13.4 at eighty minutes and both obstetric patients at 42.5. At two hours the normal stood at 17.6 and the obstetric patients at 47.7 and 48.0.

Chemical determinations have been run on both active and inactive cases. The nitrogen values were disregarded after normal figures had been found in the earlier cases. Study of the inorganic constituents was continued for a longer time and determinations have been made in whole or in part on twenty two patients. Chlorides have been determined in twenty patients. The amount in the whole blood (calculated as NaCl) ranged from 310 to 594 mg per 100 cc of blood the average for the whole series being 501 mg. Calcium varied in fourteen cases from 6.0 to 11.8 mg with an average of 8.33 mg. Inorganic phosphorus in eighteen cases of varying age ranged from 2.04 to 6.45 mg. We have been unable to establish any relationship between the degree of anemia or the severity of complaint and the level of these blood constituents. There is on the other hand a marked disturbance of the cholesterol. Thirteen determinations have been made by the method of Bloor. The lowest value found was 208 mg per 100 cc of blood. Two girls each seven years of age gave identical readings of 222 mg. Both were known sicklers who had been in the hospital one ten months the other one year previously and were visited at their homes for purposes of check. Both were playing about normally and both disclaimed any complaint, although one showed a red count of 3,560,000 and a hemoglobin of 48 per cent (Sahli), the other a red count of 3,440,000 with 45 per cent hemoglobin. Four patients showed cholesterol of 260 to 273 mg three a value of 300 to 333 mg, one had 450 mg one 490 mg and one obstetrical patient who had been delivered eight days previously had a value of 877 mg. This girl was fifteen years of age had a red count of 3,390,000 hemoglobin 60 per cent, WBC 6900. Three nucleated red cells were seen in differentiating 100 leucocytes. Sedimentation rate was not taken. Included in the series just quoted were four other obstetric patients all examined at intervals of four to thirteen days after delivery and showing cholesterol readings of 266, 333, 333 and 490 mg.

We have had opportunity for making postmortem examination in four cases. A study of the first of these has already been reported.¹¹ None of the subsequent cases have been so well developed but each has been valuable as affording means for comparative study. A detailed histologic study of these cases will be reported upon at a future date but we wish to call attention at this time to certain findings that appear to us to have a possible bearing upon the general understanding of the condition.

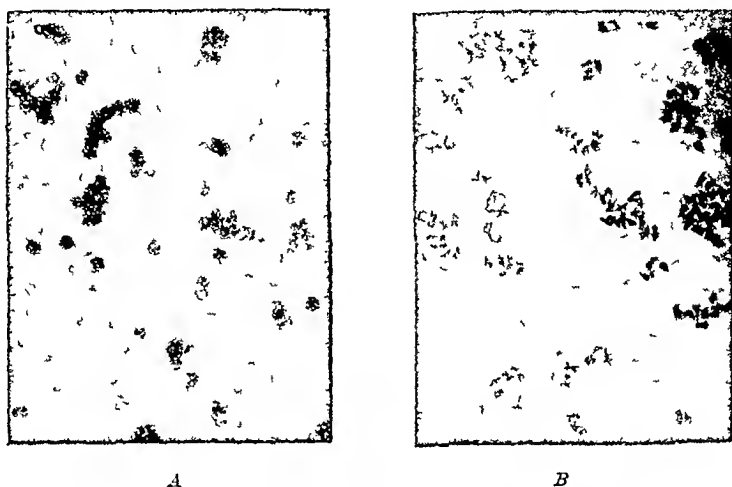


Fig 3—Cover slip preparation of blood taken from a vein during autopsy. "A" was photographed one hour after set-up. "B" about twenty-four hours later.

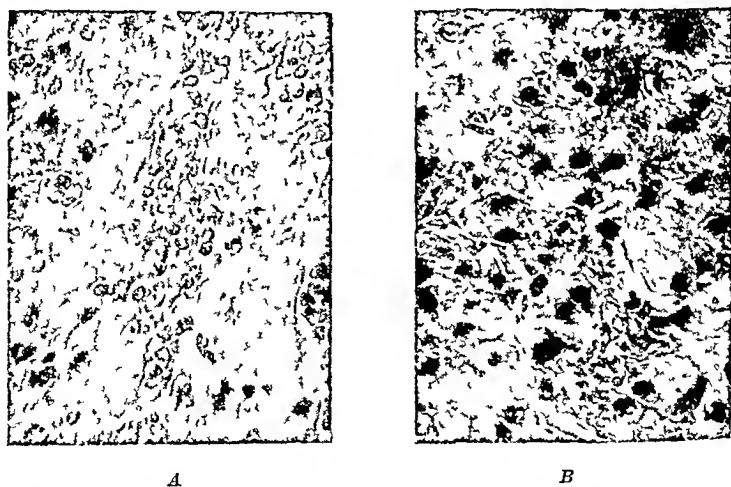


Fig 4—Sections from liver (Case II). "A" is from a block fixed in Zenker's solution. "B" from a block fixed in formalin. Both blocks were imbedded in paraffin the former by the chloroform method the latter by the simpler benzol method.

Of the four cases in the series, the first two had a similar clinical course. In Case I, a male, aged thirty years, death was due to streptococcal bronchopneumonia with associated bacteremia. Case II was that of a male, aged nineteen years. Death was again due to acute bronchopneumonia. In this case, as in the first, the lungs showed many small abscesses developing in

areas of peripheral infarction. Streptococci were present in these lesions, as well as in the pleural cavity. The autopsy was performed twenty seven hours after death. Blood taken from a vein during autopsy and sealed beneath a cover slip in the usual manner showed at first only occasional deformed red cells but next day there was typical sickling deformity in about 75 per cent of the cells (Fig 3). The same observation had been made in the first case. As in the first case also, it was found that paraffin sections of tissue fixed in Zenker's solution contained red cells of natural appearance, whereas parallel blocks from formalin fixed tissue showed well marked sickling deformity or at least an equivalent distortion of the cells (Fig 4). Case III was that of a truck driver, aged twenty nine years who died of meningeal hemorrhage three days after the receipt of accidental cranial injury. The sickling deformity of his red cells was discovered only as the result of the routine examination that was being practiced on unselected patients. He had a red count of 4,360,000 and a hemoglobin of 90 per cent the highest values for any patient in our series. In Case IV the age was twenty three years. The patient had a positive Wassermann and cutaneous scars of a secondary luetic eruption. He died of peritoneal and generalized tuberculosis after an illness of about three months duration.

The one finding common to all these cases was as might be expected the evidence of a continued erythrocytic destruction rate higher than the normal. Such evidence consisted, first in the widespread deposit of hemosiderin in the tissues, most marked in the spleen but present also in liver, bone marrow and lymph nodes. There was also definite hyperplasia of the bone marrow in the first two cases. The marrow was not obtained in Case III. In Case IV, the marrow of the femur showed well marked serous atrophy. In all four cases many phagocytic cells containing red cells or blood pigment were found in the great reticuloendothelial centers and such cells occurred also in occasional vessel lumina of various organs.

DISCUSSION

Following present usage we have labeled as sickle cell anemia every case in which sickling deformity has appeared in the red cells. But as knowledge of the condition increases it will probably become advisable to restrict the use of this term. In some of our cases there has been no clinically recognizable evidence of anemia at the time of examination. We believe that in the present state of our knowledge it would make for clarity were we to look upon this cell deformity merely as one demonstrable stigma of an inheritable blood dyscrasia or status upon the basis of which there may under conditions not yet understood, be built an anemic syndrome of well characterized type. If the problem be thus conceived there is need for some word that may serve to designate the presence in a given blood of the sickling anomaly without at the same time committing us to any decision on the further question as to what other morbid changes may have made their appearance in the given case. To fill such need we have devised and would offer for adoption the term "menisocytosis." It is derived from the Greek *meniskhos*, "meaning "a sickle." As used, it would designate a peculiar and apparently

unique type of poikilocytosis dependent on familial and probably on racial abnormalities of the red cells or of their suspending fluids or of both, partly inherent in the fresh blood cells but fully developed only under artificial conditions. We believe that the occurrence of this meniscoctosis in the blood of a given individual need not necessarily imply the presence of any clinically demonstrable anemia but it should at least serve as a warning signal. The affected individual is at once included in that 5 to 7 per cent fraction of the population in the negro wards which deserves special care in treatment and prognosis. As stated above, these individuals appear very susceptible to infectious disease and particularly to infections of the respiratory tract. Among the patients of the present series there has been a high incidence of tonsillitis, bronchitis and pneumonia. Of four deaths in affected persons, two were due to bronchopneumonia. The condition is of serious import in children, as was pointed out by Sydenstricker in his first paper. In the present series of cases from the wards of the Hillman Hospital, a charitable institution accepting everything except contagious diseases, 50 per cent of the patients have fallen in the age group of twenty one to thirty years. The condition would appear also to be of serious consequence to the young adult.

We have as yet no explanation for the meniscoctosis. The freshly drawn blood may show abnormalities in the size, shape and staining qualities of the red cells. These may become so marked as to be diagnostic, particularly when, as is not uncommon, they are accompanied by erythroblasts. But on the other hand they are often so slight as easily to escape notice. Yet in the latter case, as in the former, sealed preparations may develop extreme deformity. The phenomenon is probably dependent on a peculiar lability of the erythrocytic cytoplasm. It may be influenced readily by variations in the physical conditions to which the cells are subjected *in vitro*, as is evident from the above note on the different reactions obtained through variation in the cover glass thickness. Such differences probably depend upon the securing of optimum pressure conditions. It is probable that another factor in the production of distortion is the lateral traction exerted upon the individual cells by adherent fibrin filaments formed during coagulation. Neither force is potent when acting alone. Together they produce distortion. Red cells acted upon within the tissues by fixing fluids may preserve their rounded shape in the presence of a "good" cytologic fixative such as Zenker's solution but may suffer distortion under the unequal strains produced by the shrinking action of a "poor" fixative such as formalin.

Biochemical factors may contribute to the erythrocytic abnormality that makes deformity possible. The red cells of these individuals exhibit a striking rouleau formation and there is often a distinct granularity in cover slip preparations with the appearance of clumps in which the rouleaux are irregularly formed or heaped together. When the cells are separated from their plasma or serum and subsequently remixed, this granularity becomes more marked, particularly when the materials have been chilled and the preparations are kept at low temperature. Sydenstricker¹² states that he has observed undoubted autoagglutination in four cases. No very clear-cut results have been

obtained by previous workers through crossing red cells and serum or plasma of normal and of affected individuals nor have the few trials made by us along this line been entirely decisive but it is probable that in the 'sickler's' blood there is abnormality both of the cells and of the plasma bodies. In our first case, we were impressed by the patient's repeated statement that he was particularly liable to periods of illness after he had been exposed to cold or dampness and that his brother suffered similar disturbance under like conditions. His fatal seizure began the day after a snowstorm during which he had become cold and wet. There was here a distinct suggestion that the attacks were of a nature similar to those of paroxysmal hemoglobinuria. But the latter disease may occur in the negro without the appearance of malarial coezytosis, as we had opportunity to observe while the study of this first case was in progress. The failure to find the red cell anomaly in a condition marked by such profound disturbance of the serum immune bodies as is present in that disease lead us to doubt the influence of such bodies on the sickling deformity and to prefer to it the hypothesis that the influence of exposure might more simply be explained as predisposing to infection and that infection merely emphasized a latent blood anomaly. But as we have had from an occasional patient the repetition of this belief in the predisposing action of exposure and particularly as we have been more impressed by the observation of the pseudoclumping if not actual autoagglutination in repeated specimens of fresh blood, we have been inclined to return to the earlier idea that serum anomaly may be present and significant. It must still be remembered that autoagglutination will not explain all the phenomena of the condition. We have recently had under observation a white woman whose blood contained autoagglutinins. She suffered a long and difficult convalescence from puerperal septicemia and exhibited a profound anemia but her red cells never showed any evidence of malarial coezytosis.

In our present series of four autopsied cases the histologic finding that seems to be most constant and most significant is the widespread occurrence of a rather active phagocytic destruction of the erythrocytes. It is prominent even in Case III that of a truck driver apparently healthy and possessing a normal red count and hemoglobin who died three days after the receipt of accidental cerebral injury. It will be recalled that endothelial leucocytes containing red cells are common in the circulating blood during life. They were first noted by Emmel. Sidenstricker states that they may be found in every case if carefully looked for. Judging from the number of these cells found in sections of the liver, spleen, bone marrow and lymph nodes, as well as in occasional vessel lumina of various organs, the actual number of erythrocytes constantly in process of destruction must be considerable. This finding recalls the condition that has been described for hemolytic jaundice as well as the marrow changes in pernicious anemia discussed recently by Peabody.¹⁵ We were first inclined to look upon this phagocytosis as evidence of infectious injury of the red cells. A streptococcus had been cultured from the heart blood of our first case and great numbers of chained cocci were seen in sections from the pulmonary lesions. The pulmonary changes in Case II were very similar to those of the original case

and like them contained a streptococcus. In Case IV death was due to tuberculosis. But in Case III there was no evidence of infection yet here also there is active phagocytic destruction of the red cells in the great reticulo-endothelial centers. Despite the presence of meniscocytosis this patient presented no evidences of "sickle cell anemia." It would appear, then, that phagocytic destruction of the red cells may occur in the absence of any evident infection and that it may also occur without producing any of the usual clinical evidences of anemia. Meniscocytosis and endothelial erythrophagocytosis constitute, perhaps, anatomic expressions of a fundamental blood dyscrasia accompanied by or dependent upon the presence in the plasma of a body mimical to its own red cells. The hypothesis offers a rational basis for the explanation of the unquestioned inheritability of the condition. Given an individual thus constituted, we may explain such phagocytic destruction of the red cells as is found in Case III, we may explain also the hyperplasia of the bone marrow observed in our cases and reported also by Svdenströcker and by Hnek. Such hyperplasia may compensate for the constant drain upon the formative cells and so maintain normal values for the circulating blood. But in some cases it is unable to do this. The individual must then accustom himself to a lowered erythrocytic volume. He is handicapped under conditions of special stress and falls ready prey to the attack of toxic or infectious noxa. The surprisingly high hospital admittance rate for individuals in the third decade of life may indicate that at this age period the bone marrow begins to fail under the burden of its years of work hypertrophy. So we find in Case IV the gelatinous marrow of old age or long continued exhaustion making its appearance in a young man. The fatal illness in this case would hardly be expected to produce such extreme exhaustion of the marrow under ordinary conditions.

The increased cholesterol content of the blood is of considerable interest. Several of our high values were obtained in puerperal women, but similar figures were found in other adult patients, and also in children who were free of complaint although definitely anemic. This high level contrasts sharply with the lowered values common in other anemias and particularly with that found in pernicious anemia. It might be suggested that in view of the anti-hemolytic property of cholesterol the increase in its amount in the blood may represent some reaction of defense against the active erythrophagocytosis. The validity of such a theory would, however, be raised in question from the fact that an argument essentially the opposite has been advanced to explain the increased blood destruction in pernicious anemia.

The general relationship of sickle cell anemia to other diseases of a similar nature is not yet established. Under the designation of "Henrick's Anemia" Ward has suggested the inclusion of the disease as one type form in his "Anemias of the Hemolytic Jaundice Group."¹¹ His paper develops the conception of a group of diseases dependent fundamentally on a congenital or hereditary plasma defect. In at least some members of the group the abnormality of the plasma consists in the presence therein of an auto-agglutinin. Whatever its nature, the plasma defect renders the red cells "more prone to destruction, either by the normal mechanism of portal

hemolysis, or by phagocytosis, or by both." In hemolytic jaundice there is an almost purely hemolytic anemia, in "Mallin's syndrome," an almost purely phagocytic anemia, while both types of cell destruction are present in Herrick's anemia. Outside hemolytic jaundice, Ward has been able to collect but few cases illustrative of his type forms but his conception is interesting. Certainly, in the light of our present knowledge Herrick's or sickle cell anemia seems, despite its divergences, more closely allied to hemolytic jaundice than to any other commonly recognized syndrome.

REFERENCES

- ¹Herrick J B Arch Int Med 1910 xi 517
- ²Washburn R E Virginia Med 1911, vi 130
- ³Cook J E and Meyer, J Arch Int Med 1915 xii 644
- ⁴Mason, V R Jour Am Med Assn, 1922 lxxix, 1718
- ⁵Dresbach, M Science, 1904 xix 469 ibid 1905, xix, 417
- ⁶Bishop, F W Arch Int Med 1914 xi 388
- ⁷Emmel, V E Arch Int Med, 1917, xx 586
- ⁸Sydenstricker V P Mulherin, W A, and Houseal, R W Am Jour Dis Child, 1920 xxvi, 132
- ⁹Huck J G Bull Johns Hopkins Hosp 1923 xxxv, 335
- ¹⁰Sydenstricker V P South Med Jour 1924, xvi 177
- ¹¹Gritham, G S Arch Int Med 1924 xxxiv 7,8
- ¹²Sydenstricker V P Jour Am Med Assn 1924, lxxxi 12
- ¹³Penbody, F W and Brown, G O Am Jour Path 1925 i 169
- ¹⁴Ward, Gordon Proc Roy Soc Med, 1919 20, xii Pt 1 and 2, Sec Med, pp 1 21

A COMBINED DILUTING AND STAINING FLUID FOR DIFFERENTIAL LEUCOCYTE COUNTS IN THE COUNTING CHAMBER*

BY DANIEL NICHOLSON,† WINNIPEG, CANADA

THE most widely used method for differential leucocyte counts is the spreading of a drop of blood on a glass slide which is examined under the microscope. A great concentration of polymorphonuclear cells will be found at the margin and at the end of the smear. Many authorities recommend examining in a zigzag manner across the slide so as to take in the polymorphs at the edge and lymphocytes in the center of the smear. No suggestion is made regarding how far one should go into the body of the smear where most lymphocytes are met. A worker who examines half an inch into the body of the smear will obtain a higher lymphocyte percentage than one who examines in only a quarter of an inch. Some recommend examining right across the slide. By any of these methods the accumulation of polymorphonuclear cells at the end is left out entirely so that an accurate differential count is impossible. In the cover glass spreads the distribution of the leucocytes is more uniform but for some reason, perhaps because of technical difficulties, this method, although it originated many years ago, has never been generally adopted.

The cells in the counting chamber are evenly distributed and if a satisfactory stain is mixed with the diluting fluid one can distinguish the different forms of leucocytes and so make a differential count.

Dunger used $\frac{1}{2}$ per cent eosin in 10 per cent acetone to stain the eosinophiles in the counting chamber. Later Dr. Stitt in the sixth edition of his excellent textbook on practical bacteriology used Giemsa's stain added to a weak neutral formalin solution, as a combined diluting and staining fluid for leucocytes. With this, the leucocytes were well stained but had a fuzzy outline and the red cells were only partially laked. The procedure is omitted in the seventh edition of this textbook.

Frequently a clinician requires a total and differential leucocyte count from the one patient without any information regarding the red cells. By the counting chamber method we could make a total count of their number per cubic millimeter and by examining fifty microscopic fields under the high power lens, an accurate estimate of the various types of cells could be made. Thus the total and differential leucocyte count could be done in less than half the time required by our present methods.

There are many difficulties in obtaining an ideal combined diluting and staining fluid. There must be no clotting or clumping of the blood. The red cells have to be hemolyzed or rendered transparent. The fluid must

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas Texas April 15 16 and 17 1926

†Assistant Professor of Pathology University of Manitoba Winnipeg Canada.

hold the stain in solution in sufficient concentration that it will stain the cells in a few minutes in such a manner that the various types may be recognized. It must have a specific gravity less than the cells or they will float about instead of resting on the bottom of the counting chamber. Each one of these requirements can be readily obtained singly but I have spent many hours indeed trying to find a fluid that would combine all these features.

The most convenient and material saving technique I have found is. Place a large drop of the diluting fluid on a glass slide under the low power of the microscope. Add a small drop of blood by means of a platinum loop and notice if there is any clumping of the cells, coagulation of the protein or hemolytic bleaching action on the red cells. If no clumping of the cells or clotting of the protein takes place and the red cells become invisible but



Fig 1—Photomicrograph of the end of a blood smear. Note the large collections of polymorphs which appear as small dark circles and the scarcity of these cells farther in.

the leucocytes remain uninjured, place two more large drops on a glass slide. To one add a small amount of nuclear stain by means of a toothpick and note the intensity of the solution seen under the microscope as a test of solubility. Treat the second drop with a cytoplasmic granular stain such as eosin or acid fuchsin and observe in the same manner. Mix a part of each drop and if a precipitate occurs it can be readily seen under the microscope. To all three drops add a loopful of blood and observe the degree of staining under the microscope. I tried all the possible combinations I could think of in this manner. This technique is very simple, rapid and satisfactory. If clumping did not take place and some staining of the cells occurred, I made $\frac{1}{10}$, $\frac{1}{4}$, $\frac{1}{2}$ and 1 per cent dilution of the powdered stain in the diluting fluid and used it to dilute the blood in the leucocyte pipette. Here I might mention that if one is not using a dilution pipette of this type constantly it is difficult to draw the blood up to the correct mark and no farther. If it

is drawn up farther and blown back, a coating remains on the tube which makes for inaccuracy. I have spent considerable thought on a pipette to overcome this and of course a syringe arrangement suggested itself very soon. I found out later that Pappenheim had brought one out on this principle in 1910. It was not satisfactory because the bore of the pipette is so small compared with that of the syringe that, with the slightest syringe movement, the blood moves quite rapidly and is liable to pass the mark. To overcome this, I devised a bevelled lower end of the syringe so that by turning it, a very slight backward movement, just enough to draw up the column of blood slowly, is obtained. For the diluting fluid the syringe may be drawn back in the ordinary way. The error of not being able to stop exactly at the upper mark causes only one-twentieth the error of a miss at the lower mark.

With the diluted blood in the pipette, after one minute's shaking I

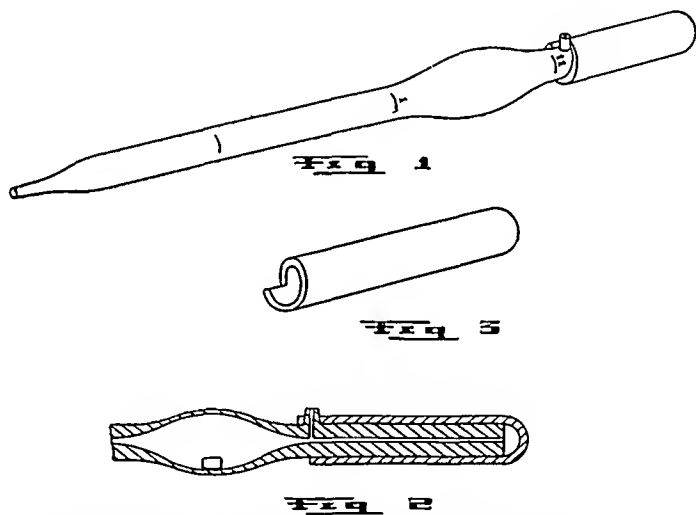


Fig. 2—Precision leucocyte pipette. Fig. 1 Assembled. Fig. 3 The cap with beveled proximal end which when turned produces a slow and steady backward motion. Fig. 2, a cross section of the pipette. To obtain a vacuum the thumb must be placed on the knob while the cap is drawn back.

placed a drop in the counting chamber and examined it under the microscope at intervals of three, five, ten, and fifteen minutes from the time of filling the pipette, recording the results in each case.

The best hemolytic substances I found were saponin and sapotoxin but the stains dissolved in them did not produce a clear-cut outline of the cells. All the benzine substances were tried but as they were very poor stain solvents they had to be given up. Watery solutions did not hold many of the stains well and any substance which merely produces a rupture of the envelope of the red cells was unsatisfactory because the ruptured fragments partly stained, were strewn about the microscopic field and obscured the view of the leucocytes.

The most satisfactory type of fluid is one which dissolves out or renders the pigment in the red cells transparent. Acetone will do this and will mix with water. A 10 per cent watery solution will make red cells transparent.

but over 25 per cent will coagulate the blood. It will not hold the eosin methylene blue stains like Wright's and Jenner's stain solution, in sufficient concentration to dye the cells but will hold Giemsa's stain. The prepared stain contains alcohol and glycerin which retard the action of the acetone on the red cells so that it is necessary to make an increase of the acetone up to 20 per cent to obtain the desired bleaching on the red cells. When these solutions are mixed a slight precipitate occurs which does not interfere with the examination but may be removed by decanting the supernatant fluid one hour after the stain is added to the acetone solution.

Of all the combinations of diluting and staining fluids I have tried 6 per cent Giemsa's stain in 20 per cent acetone is the most satisfactory. Keep the

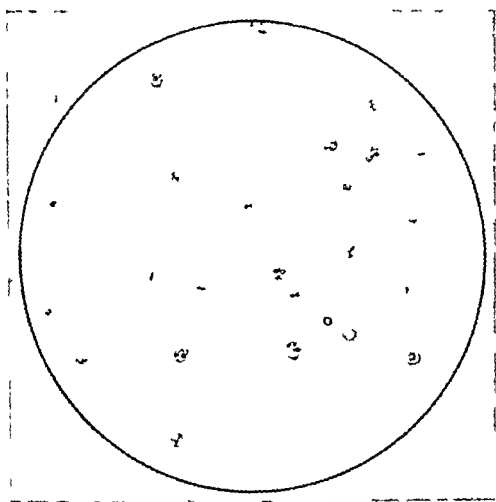


Fig. 3—Appearance of white cells in the counting chamber under the high power lens when 6 per cent Giemsa's stain in 20 per cent acetone is used as a diluting fluid.

pure Giemsa's stain in one bottle and 20 per cent acetone in distilled water in another. Before making a count add 0.6 cc of Giemsa's stain to 10 cc of the 20 per cent acetone.

As a diluting fluid it renders the red cells transparent in five to ten minutes. This can be determined by watching a change in the bulb of the pipette from opaque to transparent. After shaking the pipette a drop of the contents is placed in the counting chamber and examined under the high dry lens with a full light and the condenser slightly down. That most important part of the cell the nucleus stands out very clearly. One can readily differentiate the young, solid U-shaped nucleus from the mature segmented ones. This is important in the Arnet or the modified Schilling counts. The mononuclears show a

very faint cell outline The eosinophiles can be distinguished by their red dish cytoplasm Crystals of the stain are seen but they do not obscure the view of the cells The blood platelets are faintly stained and may be seen in clumps The fluid will keep one day, after which time it stains more slowly

It is not without its faults and drawbacks however At least five minutes are required for the solution of the red cells and the staining of the white cells The pipettes and counting chamber must be scrupulously clean If the faintest trace of acid reaches the solution the red cells take on a pinkish stain and the nuclei of the leucocytes are very faint This is the commonest cause of failure as frequently a trace of acetic acid remains in the pipette This acid reaction can be recognized by noting a change in color of the diluting fluid from deep purple to a greenish blue in the bulb of the pipette

If the faintest trace of alkali becomes mixed with the fluid, the granules of the eosinophiles will be greenish or if the alkali is strong they will remain altogether unstained The acetone evaporates more rapidly than a watery solution would, but a total and differential count can be easily done before the evaporation will impair the result The solution is not a permanent one The Giemsa's stain must be added to the 20 per cent acetone solution daily

It would be an important advance to obtain a combined diluting and staining fluid that would not require such exacting precautions While we are waiting for this, the difficulties mentioned can with a little care be overcome and one can make a reliable differential leucocyte, or modified Arnet count almost as quickly as doing an estimation of the total leucocytes

SUMMARY

1 The leucocytes are not evenly distributed on a glass slide and it is impossible to do an accurate differential count of them by this method

2 The leucocytes are evenly distributed in the counting chamber and if 6 per cent Giemsa's stain in 20 per cent acetone is used as a diluting fluid the red cells are rendered transparent and the leucocytes well stained so that a differential count can be made under the high power lens

3 Even faint traces of acid or alkali interfere with the staining

4 A fresh mixture of Giemsa's stain and acetone solution must be made daily

DISCUSSION

Dr A H Sanford—Dr Nicholson asked me to open the discussion, but after reading and hearing his paper I feel there is very little to say, as he has covered the ground so thoroughly He has pointed out the needs of the methods, and also the difficulties encountered

There is one matter that might be mentioned relative to pipettes For a number of years I have been interested in improvements of hemocytometer pipettes The pipette made for A H Thomas Co according to the design of Mr Tierner is attracting attention Recently I have also received from England an automatic pipette, the Piney pipette, made by Hawskley & Sons, London, England, which is apparently very accurate

Dr Nicholson's ingenious method is worth a trial by all of us

Dr B F Stout—I have for many years felt little confidence in differential counts made on slide smears The Germans have always insisted that cover slip spreads be used, and some writers in this country prefer their use I have for years where a critical differential count was necessary, made the count of the percentage of polymorphonuclears in the

counting chamber. Here the best distribution of cells may be found. The nuclei are readily distinguished by the high dry power with the dilute acetic acid and careful counts with this method have shown the inaccuracy of the slide-smears. I am very much pleased to have heard Dr Nicholson's paper and feel sure it is an important advance in the interests of accuracy as well as a time saver.

Dr F W Hartman.—This method is very interesting. I think this Society could well emphasize, as a Society, some of the points Dr Nicholson has made in regard to these counts.

Why is it that 90 per cent perhaps 95 per cent of our students count by this slide method? Why are they not shown the cover slip method? When they do know the cover slip method they do not use it.

How does this work on spinal fluid? It sounds as though it might work. I would like to know how long the cells will keep in the pipette. Could we draw the blood this morning and several hours later do the count and get satisfactory results?

Dr Otto Lowy.—It seems to me that in the discussion some stress was laid upon whether it would be easier to do this than the slide method. I think that in our organization we should place accuracy above rapidity. That is about all I have to say. I wish to complement Dr Nicholson on his work.

Dr Philip Hilkowitz.—Sir Almoth Wright in his book *The Technique of the Test and Capillary Glass Tube* emphasized the inaccuracy of the usual slide method because the polys would be unequally spread if the spreader were not perfectly plane. He has developed a technique whereby the smears are made uniform. He takes a slide, splits it in two and measures carefully that particular slide. It has to be perfectly plane. One sometimes has to examine perhaps a whole gross of slides before finding one that is suitable. Once that ideal spreader is found it is employed for making all smears, thus doing away with the defects of having an uneven smear. There is another item to be taken into consideration in blood counting, and that is the question of rapidity. It is a matter of importance in hospital work where a great many examinations have to be made. To insure that the work is turned out quickly and efficiently the method has to be simple and easily taught to the technician. Any method that will complicate the matter will not be feasible. If as Dr Nicholson proposes it would be at the same time accurate and easy to carry out then it would certainly be an advantage to use this method.

Dr Nicholson (closing).—Differential counts in the counting chamber and cover glass preparation do not vary very much if many cells are counted on the cover glass. There is a much greater variation in counts done by the slide method. Frequently with good technicians the polynuclears will vary 20 per cent. With any method we have the difference in classification. Some will call the lymphocyte with a moderate ring of cytoplasm, a large mononuclear; others will call the young form of polymorph with solid nucleus, that is well indented, a large mononuclear and so forth. If one could find a fluid that would produce an oxidase reaction this difficulty would be largely overcome. The fluid works very well in doing a cell count on spinal fluid. In blood counts done away from the laboratory, the cells show well after the diluted blood remains in the pipette three or four hours. After that time the cytoplasm of the cells becomes rather deeply stained.

ON THE TOXICITY OF TETRAETHYL LEAD AND INORGANIC LEAD SALTS²

BY ROBERT A. KEHOE, M.D., CINCINNATI, OHIO

I INTRODUCTION

IN THE course of studies on lead poisoning the toxicity of tetraethyl lead has been determined in the case of rabbits, and a comparison has been made with the toxicity of other and commoner lead compounds. The details of the method of study are included herein, and in addition to the data on the toxicity, certain important actions of tetraethyl lead, are presented.

1 It is capable of direct absorption in lethal quantities through the intact skin of rabbits.

2 Its toxicity varies little from that of other and water soluble lead compounds. This fact indicates that its toxicity is a function of the lead, and not of any peculiar qualities characteristic of the compound.

II MATERIALS

Commercial tetraethyl lead was carefully purified by steam distillation to water white clarity and constant specific gravity. The other chemicals were the best quality, chemically pure materials obtainable.

The animals were healthy rabbits of various ages and sizes, from complete maturity down. They were kept under the best possible laboratory conditions as to food and hygienic conditions.

III EXPERIMENTAL METHODS

The toxicity of tetraethyl lead was determined on the basis of intravenous, oral, cutaneous and respiratory administration. It was prepared for intravenous injection by dissolving it in sterile cotton-seed oil, so that 1 cc represented 0.04 cc tetraethyl lead. Injection was made very slowly so as to avoid gross pulmonary embolism.

Cutaneous administration was made after carefully clipping the hair from the belly, without injury to the skin.[†] The animal was then placed in a hood, with the head directed into the source of air supply, and strong ventilation was employed until the treated area appeared to be dry. This was found to require at least an hour. The animal was then removed from the hood and put into a well ventilated cage for observation.

The material was introduced into the alimentary tract by dropping the

²From the Eichberg Laboratory of Physiology, University of Cincinnati, Cincinnati, Ohio.
Received for publication September 24, 1926.

[†]It was found inadvisable to shave the skin because of the likelihood of slight injuries and more important because moistening and soaping the skin were found to interfere seriously with absorption.

required amount directly into the open mouth of the animal. No difficulty was experienced in causing the animal to swallow the entire dose.

Inhalation studies were made in which air was bubbled through tetraethyl lead and mixed with fresh air, the mixture being passed through tight cages containing animals, in such a manner as to subject the animals to known concentrations of tetraethyl lead vapor. The apparatus for this purpose consisted of a metal box, the metal and glass front of which was fastened on with nuts, a rubber gasket serving to make a tight enclosure. An inlet tube was arranged in the top at one side and an outlet tube at the opposite side. Air was passed through the cage at the rate of 5 liters per minute, this air being admixed with a varying quantity of air saturated with tetraethyl lead vapor.

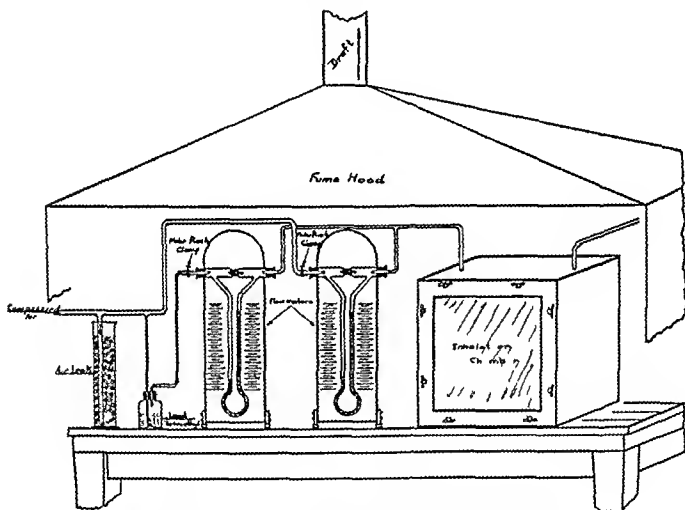


Fig 1—Arrangement of apparatus for inhalation experiment.

The volumes of fresh air and of air saturated with tetraethyl lead were measured by means of carefully calibrated flowmeters. Admixture was accomplished by joining the two tubes conveying them with a Y tube and leading them thus into the cage. Calculations of the lead concentration in the air were made from the previously determined lead content of unit volumes of air saturated with tetraethyl lead at the temperature of the experiment. The apparatus used is illustrated in Fig 1. The entire operation of this experiment was carried out in a hood with strong forced ventilation. The animals were exposed daily for a period of not to exceed six hours, no food or water being furnished them during the period of exposure.

The toxicity of lead nitrate and lead chloride for rabbits was compared with that of tetraethyl lead by their administration intravenously.

After treatment in the various ways of the experiment, the animals were observed for the appearance of symptoms. Certain signs and symptoms appeared which came to be recognized as characteristic of acute poisoning.

In case death occurred, the tissues were carefully examined for abnormalities of all kinds. Certain characteristic pathologic changes were found, these being associated at times with other lesions unrelated to those experimentally induced.

From the clinical and postmortem observations it became possible early in the course of the experiments to determine whether death of the animal had been brought about wholly by the poison itself or whether it had been caused in part by previously existing disease or experimental accident.

IV EXPERIMENTS AND DATA

1 *Intravenous Injection of Tetraethyl Lead*—A review of the protocols shows the first symptoms appearing in about an hour after the injection of lethal doses of tetraethyl lead. At first there is an increase in respiration, and the animal moves about. Then there is a slowing of the respiratory rate. At this time the animal usually sits quietly as if asleep, arousing occasionally. When he moves, muscular incoordination is observed. The general depression increases and as a terminal manifestation a slight convulsion usually occurs. Death results in from four to twelve hours, dependent upon the dose administered, respiratory paralysis being the immediate cause.

The pathologic findings are set forth in the following necropsy protocol.

Rabbit No. 27—2.7 kilo, dead ten hours after injection. The lungs are edematous and there are numerous petechial hemorrhages. No infarction is present. The abdominal viscera show general capillary dilatation. The right heart is hugely dilated, the left ventricle being contracted. The pylorus of the stomach is very strongly contracted. The upper small intestine filled with mucoid, watery, yellowish liquid, slightly blood stained in one or two coils. The mucosa is almost completely eroded. The large intestine is unaffected. The kidneys show no gross abnormality. The liver is dark red, the markings are indistinct, and there is general cloudy swelling. The brain is more than normally friable, and there is considerable excess fluid at the base beneath the dura.

The degree of injury to the organs varies with the dosage, and especially with the time which elapses between administration and death. In fact, edema of the brain cannot be detected in an animal which dies quickly. Otherwise the above pathologic findings are constant and characteristic.

Table I shows the results of varying dosages of tetraethyl lead in cotton seed oil (1 cc = 0.04 cc Pb(Et)₄) injected intravenously. When the solution is injected pulmonary infarcts are often produced. If injection is made too rapidly, large emboli bring about death quickly. If injection is made very slowly either very small infarcts or none develop, and the animal dies or survives according to the size of the dose.

TABLE I

RABBIT	WEIGHT	DOSE OF Pb (Et)	RESULT
18	1.6 kilo	0.004 cc	Survived
19	1.9 kilo	0.01 cc	Survived
20	1.4 kilo	0.02 cc	Survived
24	2.8 kilo	0.033 cc	Survived
25	3.0 kilo	0.04 cc	Death in five minutes
27	2.7 kilo	0.04 cc	Death approx. 10 hours
28	2.8 kilo	0.04 cc	Death approx. 12 hours
33	2.8 kilo	0.03 cc	Survived

Pulmonary embolism

The above data show that animals of about 3 kilo are uniformly killed by doses of approximately 0.04 cc Pb(Et)₄ given intravenously. Calculating on the basis of a specific gravity of 1.6591 for Pb(Et)₄, the lethal dose per kilo, in terms of Pb is approximately 0.014 gm.

2 *Cutaneous Application of Tetraethyl Lead*—This method of administration eliminates any factor other than the direct toxic effect of tetraethyl lead. The symptoms, however, are those previously described, plus a striking increase in peristaltic action visible through the abdominal wall within a few minutes after treatment. There is usually bladder irritation as well, small quantities of urine being voided frequently.

At necropsy the lungs are found to be normal or only moderately congested and the abdominal wall at the site of application is thick with subcutaneous edema. Otherwise the pathologic changes are identical with those produced by intravenous injection.

Table II shows the results of the cutaneous treatment of a series of animals with pure tetraethyl lead. It may be seen that there is little variation in susceptibility to a single large dose of the compound.

TABLE II

RABBIT	WEIGHT	DOSE OF Pb (Et)	RESULT
36	2.4 kilo	0.4 cc	Survived
40	2.0 kilo	0.6 cc	Survived
69	1.4 kilo	1.0 cc	Death in 24 hours
70	1.4 kilo	1.0 cc	Death in 24 hours
71	1.3 kilo	1.0 cc	Death in 24 hours
124	1.5 kilo	1.0 cc	Death in 24 hours
72	1.5 kilo	0.75 cc	Survived
73	1.6 kilo	0.75 cc	Survived
74	1.6 kilo	0.75 cc	Survived
120	2.0 kilo	1.5 cc	Death in 24 hours

It is seen from Table II that four animals weighing about 1.5 kilo died when treated with 1 cc while three animals of approximately the same weight receiving 0.75 cc survived. It is scarcely worth while to sacrifice other animals to attempt a refinement of these results. Considering the unavoidable variation in animals, and the variation in absorbing surface which must occur in such an experiment, the lethal dose for animals of this size is well defined. The above data indicate that the lethal dose of tetraethyl lead for rabbits when applied cutaneously is approximately 0.7 cc per kilo. This calculated in terms of Pb per kilo is equivalent to 0.7 gm.

3 *Oral Administration of Tetraethyl Lead*—When tetraethyl lead is given by mouth the symptoms and signs of illness and the pathologic lesions which appear are the same as those described above. The process appears to be a little slower, however. None of the animals so treated died in less than two days, and one survived for five days.

Table III shows the results of the experiments.

TABLE III

RABBIT	WEIGHT	DOSE OF $Pb(ET)_4$	RESULT
37	27 kilo	0.04 cc (in oil)	No illness
60	20 kilo	0.1 cc (undiluted)	Ill Survived
54	16 kilo	0.2 cc (undiluted)	Died in 5 days
48	18 kilo	0.3 cc (undiluted)	Died in 2 days

The lethal dose by mouth calculated from the above data is approximately 0.12 cc $Pb(ET)_4$ per kilo, or about 0.12 gm per kilo in terms of Pb.

4 *The Inhalation of Tetraethyl Lead*—Death of experimental animals from tetraethyl lead poisoning occurs most rapidly if the material is inhaled in high concentration. The saturated vapor of tetraethyl lead may produce death in two hours. The same symptoms of poisoning are seen as have been described, but they develop with surprising suddenness.

The most marked variation in pathology seen in animals treated in this way is in the nasal mucosa, which is red, swollen and covered with an adherent frothy fluid. The trachea and bronchi show no corresponding condition, and the lungs show little edema. In these animals the liver shows much less congestion and cloudy swelling, and the central nervous system is not grossly abnormal. Again the most striking lesion is found in the duodenum, which presents the same characteristic appearance previously described.

Table IV shows the results of studies in which rabbits were exposed to varying concentrations of tetraethyl lead vapor in air.

TABLE IV

RABBIT	WEIGHT	FLOW PER MINUTE THROUGH CAGE		RESULT
		PURE AIR	AIR SAT WITH $Pb(ET)_4$	
3	15 kilo	none	5.0 L	Death in 2 hours
6	12 kilo	5 L	0.5 L	Death in 6 hours
11	16 kilo	5 L	0.25 L	Death 3rd day
16	33 kilo	5 L	0.20 L	Death 3rd day
38	34 kilo	5 L	0.15 L	Survived after 140 days
32	25 kilo	5 L	0.10 L	Died 100 days

The above table shows the concentration of tetraethyl lead vapor lethal for rabbits to be about 1 part of saturated vapor of tetraethyl lead in 26 parts air by volume. This is equivalent to 0.175 mg Pb per liter of air (1 liter of saturated vapor = 0.00456 gm Pb at 25°). The amount of lead which actually passes through the lungs of the animal under conditions which produce death in three days is not capable of accurate determination. Calculating, however, on the approximate value of 20 liters per hour air consumption, the animal was exposed to about 63 milligrams lead as Pb. One such animal contained 32.10 milligrams at death.

5 *Intravenous Administration of Lead Chloride*—The results obtained by the intravenous injection of lead salts into rabbits are quite variable. There is a considerable variation in susceptibility of the animals, as well as a degree of uncertainty as to the local behavior of the injected solution.

Symptoms of prostration usually appear quickly and the animal may die suddenly within fifteen minutes to an hour after injection. As a general thing some degree of recovery takes place only to be followed in fatal cases after a variable period of time by weakness, muscular incoordination, drowsiness, and finally death. The latter course has been taken as the indication of general toxicity of the lead compound. The sudden deaths, almost immediately after injection are the expression of some change occasioned by the intravenous method of administration. That changes in the blood may occur is shown by the frequency with which thrombosis occurs at the site of the injection, and while such injection is under way.

The pathology seen in animals dying after the elapse of several hours following the injection of lead chloride or lead nitrate is confined almost exclusively to the circulatory system and the kidneys. The heart is enormously dilated (in one case the heart spontaneously ruptured) and there is a dilatation of the capillaries with petechial hemorrhages in the lungs and kidneys. The lungs are edematous, and the kidneys usually show cloudy swelling. The intense duodenal erosion in tetraethyl lead poisoning is absent. There is considerable injection of the intestinal mucosa, however, and a watery diarrhea often gives evidence of intestinal irritation before death.

Table V illustrates the variability of results and points out approximately the lethal dose of lead chloride (PbCl_2).

TABLE V

RABBIT	WEIGHT	DOSE OF PbCl_2 (1 CC.— MG.)	RESULT
43	1.84 kilo	10 mg	No illness Survived
44	1.88 kilo	10 mg	Died in 12 hours
45	1.76 kilo	20 mg	Survived
154	2.10 kilo	20 mg	Survived
50	1.64 kilo	20 mg	Venous thrombosis of ear Survived
147	2.70 kilo	20 mg	Died in 41 hours
174	2.00 kilo	20 mg	Survived
61	1.64 kilo	30 mg	Diarrhea Venous thrombosis Survived
176	2.44 kilo	30 mg	Survived
178	2.3 kilo	40 mg	Died in 20 minutes
180	1.72 kilo	40 mg	Survived
187	1.63 kilo	40 mg	Survived
19	1.72 kilo	40 mg	Died in 14 hours
189	1.60 kilo	40 mg	Died in 24 hours
190	1.62 kilo	45 mg	Died in 65 hours
190	1.41 kilo	45 mg	Died in 3 to 4 hours
192	1.77 kilo	45 mg	Very ill Survived

There is some indication in the above table that young animals are less susceptible to a single large dose of the lead salt than are older animals. There are probably one or more other factors which influence the result and which are not fully understood. Although death occurs occasionally from smaller quantities, a dose of 0.040 to 0.045 gm of PbCl_2 is required to kill the

average rabbit of 1.5 to 2.0 kilograms. This is equivalent to from 0.020 gm to 0.030 gm per kilo, or in terms of Pb, 0.015 gm to 0.022 gm per kilo. Essentially the same results are obtained when PbNO_3 is used.

V DISCUSSION

The foregoing experiments show a close similarity in the toxicity of tetraethyl lead as compared with lead chloride, under conditions in which the amount of lead in the circulatory system is capable of accurate control, namely, intravenous administration. The similarity in toxicity is so close as to warrant the conclusion that toxicity is a function of the common constituent of the two compounds, i.e., lead. The toxicity of heavy metals has long been believed to be due to the coagulation of proteins. Tetraethyl lead has little or no such property, yet it is as toxic as lead salts which have. There is a delay, however, in the appearance of toxicity. This delay suggests that something happens to tetraethyl lead in the body which brings about a toxicity which is not originally present. There are sufficient evidences that tetraethyl lead breaks down with fair rapidity in the tissues to yield water soluble compounds,[†] which may be triethyl compounds, or inorganic salts. It is probable that its toxicity is due largely to these decomposition products and that its delayed toxicity as compared with lead salts is due to the rate of decomposition, which does not allow the development of immediately high concentrations of active lead compounds.

However, any advantages to the animal which arise from the relatively nontoxic character of tetraethyl lead as such, are almost counterbalanced by the fat soluble character of the compound, which allows its selective localization in such tissues as will be most affected by it, namely, the nervous tissues. For this reason, tetraethyl lead poisoning is, essentially, a central nervous system intoxication.

VI SUMMARY

The toxicity of tetraethyl lead has been determined for rabbits for the various methods of administration.

2. Comparison of the toxicity of tetraethyl lead with an inorganic lead salt indicates that its toxicity is a function of its lead content.

3. An explanation of the delayed effect of tetraethyl lead, as compared with salts of lead is offered in that it is suggested that tetraethyl lead owes its toxicity to a decomposition reaction which produces water soluble compounds of lead which are capable of coagulating proteins.

*The delay in toxicity is seen in the skin where a condition resembling coagulation appears several days after tetraethyl lead has been applied. Also acute symptoms develop slowly even after intravenous administration of many times the lethal dose.

†Evidences of decomposition of tetraethyl lead are seen when it is noted that lead is excreted in the urine in a form from which it is precipitated quantitatively by reagents which do not attack tetraethyl lead.

THE CHLORIDE CONTENT OF CANNED SAUERKRAUT*

BY MARIAN E STARK A B MADISON, WISC

IN CONNECTION with an investigation by Dr William S Middleton of this hospital into the therapeutic use of sauerkraut in cases of vomiting¹ a request came to the chemical laboratory to determine the chloride content of the brand of kraut used in the hospital and of one or two others on the local market, for comparison. No figures seemed to be available on this subject. When we had worked out a suitable technique to meet this rather unusual request, we found such a surprising similarity in salt content of the first three samples tested that we became interested in systematically studying enough more brands to determine whether this similarity was accidental or whether canned sauerkraut conforms to a definite standard that can be depended upon for general dietary considerations.

We determined chlorides therefore in eleven different cans which included eight separate brands in five analyzing both juice and solid, and in the remainder juice only. By this time the very slight variations in figures made it appear evident that a fairly constant recipe must be followed by the canners, and since completing our study we have learned that there is indeed a federal standard regulating the proportion of salt to be put into this commodity.

In Food Inspection Decision 196 from the United States Department of Agriculture the following revised and amended definition and standard for sauerkraut was adopted by the Joint Committee on Definitions and Standards composed of representatives of the United States Department of Agriculture, the Association of American Dairy Food and Drug Officials and the Association of Official Agricultural Chemists at its meeting July 13 to 17, 1925:

'Sauerkraut is the * * * product * * * obtained by full fermentation * * * of properly prepared cabbage in the presence of not less than two per cent (2 per cent) nor more than three per cent (3 per cent) of salt * * *'

In our analyses we actually found the salt content (total chlorides expressed as NaCl) to range between about 1.5 per cent and 2.2 per cent with as great variations between two cans from different shipments of the same brands as between different brands. These discrepancies could easily be accounted for by uneven mixing and settling out in bulk handling of such materials. It was the uniformity rather than the differences that impressed us.

The analyses were made by the direct silver titration method of Whitehorn² for chloride determinations in blood and urine adapting the details to the material at hand. Juice and kraut were first separated as well as possible just by thorough draining with the kraut pressed down under a plate.

From the Sarah Workman Laboratory of Physiological Chemistry in the State of Wisconsin General Hospital, Madison, Wisconsin.
Received for publication November 4 1926

TABLE I
CHLORIDE CONTENT OF CANNED SAUERKRAUT
Three Brands from Nationally Known Distributors, and Five
from More Local (Wisconsin) Packers

BRAND	WT AND VOL OF JUICE	WT OF SOLID	TOTAL WT OF CONTENTS	PROP BY WT OF JUICE	PROP BY WT OF SOLID	CHLORIDES, EXPRESSED AS NaCl		
						JUICE		SOLID
						g/100 c c	g/100 g	g/100 g
F, (a)	168 g	615 g	783 g	21%	79%	1 865	1 804	1 637
	163 c c							
	307 g							
F, (b)	298 c c	461 g	768 g	40%	60%	1 930	1 871	1 774
	311 g							
R	303 c c	544 g	855 g	36%	64%	1 902	1 855	(a) 1 761
	323 g							
B	316 c c	592 g	915 g	35%	65%	1 799	1 759	(a) 1 605
	389 g							
R ₁ ,* (a)	380 c c	566 g	955 g	41%	59%	2 021	1 974	1 947
	258 g							
R ₂ , (b)	249 c c	696 g	954 g	27%	73%	2 124	2 053	
	389 g							
S *	380 c c	602 g	991 g	39%	61%	1 757	1 714	
	217 g							
Sn	210 c c	595 g	812 g	27%	73%	1 930	1 867	
	211 g							
S G, (a)	205 c c	361 g	572 g	37%	63%	2 228	2 167	
	193 g							
S G, (b)	188 c c	369 g	562 g	34%	66%	1 638	1 596	
	243 g							
M P	238 c c	570 g	813 g	30%	70%	1 502	1 469	
Averages						1 881	1 830	1 736

*Fading end-point encountered in titration See text

as would be done in kitchen handling. The juice was centrifuged at about 3000 R P M to remove gross debris, and duplicate portions diluted to contain a chloride concentration found, by trial and error, to be in the range expected for blood filtrates. The titration as for the latter could then be employed unchanged. The solid kraut was extracted with water as thoroughly as possible. Weighed portions were first ground to a pulp in a mortar with a little water, and the mass washed repeatedly, the washings being collected through a filter, with the aid of gentle suction into a volumetric flask of appropriate capacity for final dilution. The same dilution could be used for all the samples, both juice and solid, throughout. Preliminary tests had indicated that not enough protein material was present to interfere with the titration.

Each figure for chlorides in the table represents the average of at least two titrations. Duplicates agreed in all cases but one within a variation of 0 to 0.02 c c, which represents a maximum deviation of 0.7 per cent. By duplicates are meant determinations on separate dilutions, for the juice, but for the solid, titrations of duplicate aliquots of the diluted washings from the same original weighed sample. In two cases, in addition, separate weighed samples were determined from the same can, and these agreed within 2.3 per cent and 1.2 per cent, respectively, of each other. The inherent possibilities for error in the sampling of the solid materials are obvious.

End points in the titration were as sharp and definite as the method usually gives, with the exception of the two brands noted with asterisks in the table. In the case of "Ri" an excessive turbidity of the juice, the odor and the degree of softness of the cabbage pointed to either a greater thoroughness of fermentation, or an unusual strain of fermenting organisms. It was chiefly the juice of the first can of this brand that was troublesome to titrate, but the average of eight different determinations (maximum discrepancy 27 per cent) happened to give a figure very similar to that obtained, with no such difficulty, on a second can of the same brand from a different lot. With the other can marked there was a slight tendency to fading end point, but four duplicates agreed very closely.

The figures in the Table for the amounts of juice and solid and their relative proportions in each can are presented as of incidental interest and expressed in round numbers.

SUMMARY

Analyses of the salt content of canned sauerkraut gave values as follows for total chlorides expressed as NaCl:

For juice (eleven cans including eight brands) In grams per 100 c.c. average, 1.881 maximum 2.228 minimum 1.502 In grams per 100 grams (per cent) average 1.830 maximum 2.167 minimum 1.469

For solid kraut (seven samplings from five cans including four brands) In grams per 100 grams (per cent) average 1.736 maximum 1.947 minimum 1.605

REFERENCES

- ¹Middleton Wm S. Wisconsin Med Jour November 1926 xxv 554
 Food Inspection Decision 196 from the Dept of Agriculture Pamphlet 58270 25 from the U S Government Printing Office 1926
²Whitcomb J C J Biol Chem 1921 xlv 449

A NOTE UPON COMPLEMENT FIXATION IN TUBERCULOSIS*

BY ISAMU OGAWA M.D. MUKDEN JAPAN

THE complement fixation reactions reported upon in this communication were conducted according to the technique of the new Kolmer complement fixation test for syphilis.¹ The qualitative method was employed using 0.1 c.c. of serum and 0.3 c.c. of pleural fluid (both heated at 55° C. for fifteen minutes) with several different antigens. Each antigen was titrated for anti-complementary activity and used in an amount corresponding to one third of their unit, the technique employed in all titrations and complement fixation tests being exactly as described by Kolmer, including an antish sheep hemolytic system with titration of hemolysin and complement, a primary incubation of eighteen hours at 6 to 8° C. followed by ten minutes in a water bath, etc.

From the Japanese Red Cross Hospital Mukden.
 Received for publication October 12 1926

The first part of the study was conducted in Berlin (Prof Heyman's clinic) with Petroff and Besredka antigens, and it may be well to note in passing that the German literature contains but few references to the complement-fixation reaction in tuberculosis although that by Rabinowitsch Kempner would appear to express the consensus of opinion, namely, that the tuberculosis complement fixation is a practical and specific means for diagnosis in that a positive reaction indicates the presence of an active lesion while a negative reaction may occur in healed and clinically latent cases

The second and larger part of the investigation was conducted in the Japanese Red Cross Hospital of Mukden employing the Kolmer and Besiedka antigens

The serum of experimentally infected guinea pigs was used in addition to a large number of serums from tuberculous and nontuberculous human subjects. As stated three antigens were employed, namely, those prepared after the methods of Besredka, Petroff and Kolmer and one of the interesting phases of the study was a comparison of them

RESULTS OBSERVED IN BERLIN

Guinea pigs were inoculated with various acid-fast bacilli including different strains of *B. tuberculosis* and saprophytes. All were killed in twenty to seventy days and the serum tested. Petroff and Besredka antigens prepared of human, bovine and fowl strains of tubercle bacilli yielded a high percentage of positive reactions, Kolmer antigen was not available for these tests. It was especially interesting to note, however, that animals infected with some of the acid-fast saprophytes gave no complement fixation with tuberculosis antigen

Cases of tuberculosis presenting undoubted clinical evidences along with bacilli in the sputum were classified arbitrarily in first second and third classes according to the tuberculous state, the third class being the most advanced. Out of 125 such cases belonging to all three classes, from 60 to 90 per cent yielded a positive reaction with Petroff's antigen

First class 19 tested, 17 or 90 per cent positive

Second class 54 tested, 43 or 80 per cent positive

Third class 52 tested, 22 or 61 per cent positive

Ninety-six of these were also tested with Besiedka's antigen with 67 to 85 per cent positive reactions as follows

First class 14 tested, 12 or 85 per cent positive

Second class 45 tested, 37 or 82 per cent positive

Third class 37 tested, 25 or 67 per cent positive

It will be observed, therefore, that in this group positive reactions occurred in 42 to 90 per cent of cases, the highest percentage of negative reactions being observed with the sera of those cases presenting the most advanced stages of tuberculosis and rapidly approaching death. Also the Petroff antigen proved somewhat superior to that of Besiedka

In a second group of 56 persons presenting no clinical evidences of tuberculosis, the sera of nine yielded positive reactions with the Petroff antigen

But five of these persons were known to be syphilitic and the positive reactions were in all probability Wassermann reactions with the antigen furnished by the lipoids of the bacilli. The Besredka antigen also yielded positive reactions with the sera of syphilitic patients and appeared to give this cross complement fixation reaction more frequently and more strongly than the Petroff antigen.

Of 57 patients regarded clinically as in *early* tuberculosis and without expectoration of bacilli, 19, or 33 per cent reacted positively with the Petroff antigen and 21 or 37 per cent with the Besredka antigen. In other words fully one third reacted negatively and this is unfortunate since the complement fixation test may fail, therefore, to be of material aid in the diagnosis of early and clinically obscure and difficult cases. It is to be emphasized, however, that some of those examined may not have been truly tuberculous.

RESULTS OBSERVED IN MUKDEN

Of 27 patients of advanced tuberculosis expectorating bacilli 26 or 97 per cent, yielded positive reactions with the Kolmer antigen. The one negative case was so far advanced that death resulted seventeen days after the test was made. The sera of eleven cases of this same series were tested with the Besredka antigen and 6, or 55 per cent reacted positively.

Of 111 cases regarded as tuberculous but without expectoration of bacilli, 99, or 89 per cent, reacted positively with the Kolmer antigen, 22 of these were tested with the Besredka antigen and 10 or 45 per cent, reacted positively.

Of the pleural fluids from 50 cases of tuberculous pleuritis tested with the Kolmer antigen 38 or 76 per cent yielded positive reactions. Of 68 fluids tested with the Besredka antigen, 15 or 22 per cent reacted positively.

The Kolmer antigen, therefore yielded the highest percentage of positive reactions, but that some of these may have been due to the acquisition of anti-complementary properties on the part of the antigen in transit from the laboratory in Philadelphia, is indicated by the occurrence of as high as 37 per cent of doubtfully or weakly positive reactions with the sera of persons regarded clinically as nontuberculous. In this same group the Besredka antigen yielded about 5 per cent positive reactions.

In a group of 79 cases of syphilis with positive Wassermann reactions, the Kolmer and Besredka antigens yielded from 20 to 66 per cent positive reactions, some of these persons were probably tuberculous as well but I feel quite sure that both antigens are capable of yielding cross complement fixation reactions with syphilis antibody.

REFERENCES

- ¹Am Jour Syph 1922, vi, 82
Deutsch Med Wchnschft, 1922 XLVIII 779

IMMUNIZATION OF RATS AGAINST PNEUMOCOCCUS BY FEEDING THE ACID-KILLED GERMS AND THE INFLUENCE OF THE AGE OF THE ANIMAL THEREON*

BY VICTOR ROSS, PH D, BLOOMFIELD, N J

INTRODUCTION

OUR earlier demonstration that albino rats could be immunized against intraperitoneal injections of virulent pneumococci by feeding tissues of other animals killed by the same organism,¹ or by feeding the living germ, has naturally suggested the question whether dead germs would function in the same manner. The result of two experiments in which the germs, before being used, were heated at 80° C for two hours has already been reported and showed that little or no protection resulted against an injection of living pneumococci. At that time we mentioned that we would attempt the use of HCl acid-killed germs as an immunizing agent. Several experiments, performed largely on rats and mice, in which pneumococci killed in this manner were employed, have since been completed and form the basis of this report. The influence of the age of the animal was also studied because of its importance in any practical application of the results obtained to humans.

Three experiments were done on rats. In the first two the purpose was to learn whether pneumococci killed by hydrochloric acid would serve as well as pneumonic tissue and live germs. In the third, the influence of the age of the rat was studied, the use of acid-killed germs having been found to be effective.

METHODS

Pneumococcus, Type I, was used throughout and was grown either in beef extract or beef heart infusion with or without 0.5 per cent glucose. Sufficient normal HCl acid was added to such cultures to make the final concentration N/15 or N/20 HCl. After two hours, at room temperature, the whole was centrifuged, the germs suspended in water or in 0.1 per cent gelatin solution and cracker meal added and stirred. In those cases where heat-killed germs were used, they were grown on beef heart infusion, without glucose, and after centrifugation and suspension in gelatin solution were heated at 90° C for two hours, the cracker meal was added and the whole fed. Fresh cultures were used daily. The germs from 50 cc culture were fed to each rat daily for an average period of three weeks.

Comparison of the resistance of treated and control animals was made by injecting intraperitoneally various amounts of the virulent organism, type I, in a constant volume of 0.20 cc. Controls were always injected at the same time as treated animals so that the virulence of the culture was known for the day on which the test was made.

*From the Plaut Research Laboratory, Lehn and Fink, Inc., Bloomfield, N. J.
Received for publication Nov. 3, 1926.

RESULTS AND DISCUSSION

Table I gives the results of the experiments in which rats were fed pneumococci killed by HCl acid. In it are given the date of the experiment, the number of the rat, whether it is a control or treated animal, its weight, the dose injected to test the animal's resistance and the result. It is evident, from the rapid death produced in the controls on March 21 by 10^4 cc, that a smaller quantity would also have been fatal. The two experimental rats 343 and 329 both died, due to an overdose. On the 30th the culture was weak. On April 2, 2×10^4 cc was fatal for the control but the treated rat survived this amount. On the 3rd 10^6 cc killed a control, of two immunized rats, each receiving 10^3 cc one lived. On the 5th 10^6 cc was again fatal for a control, two of four treated animals survived 10^3 cc, two died of this quantity. On the 7th, 2×10^6 cc killed a control, two experimental rats died of 2×10^4 cc, three of 2×10^3 cc and one of 2×10^2 cc. One survived 2×10^4 cc and another 2×10^2 cc. On the 9th 12th and 17th 10^7 cc killed controls and on the 15th 10^6 cc was fatal. On the 15th, all the experimental animals died, having received at least 10,000 and 100,000 times the fatal dose which was more than they could tolerate. On the other days, in spite of some irregularity in the results it is apparent that the immunized rats tolerate in general 1,000 times as large a dose as the untreated animals.

The rats, data for which are given from May 5 to June 2 form a group immunized at a different time. On May 5 1000 and 10,000 times the fatal dose did not kill the immunized animals. On the 10th, 10^7 cc killed a control rat whereas 10^4 cc failed to kill either of two experimentals. 10^3 cc was not tried. On the 14th, a control rat succumbed to 10^8 cc another survived this amount. Three treated rats survived 10^4 cc and two died of this dose, two survived 10^3 cc. The remaining tests on the 19th, 21st, 24th, 26th and 28th of May and on June 2 all demonstrate that a decided degree of immunity exists among the rats fed with acid killed germs. The results in this group of rats are more regular and show a higher degree of immunity than those of the preceding one.

In Table I, beginning with July 20, are given the results of a similar experiment in which the additional influence of age of the animal was tested. Although the two preceding experiments were done with rats varying in weight from 112 to 175 gm in the first and from 45 to 147 gm in the second, thus covering the approximate ages of one to three months, they had been performed without an attempt to observe any influence of this factor. The present experiment was carried out primarily with this object in view, feeding acid killed organisms which were now known to be as effective as living germs. One group of rats weighed between 62 and 70 gm when feeding of germs was started, the other weighed between 263 and 305 gm at the time. Although the exact ages of these groups of animals were unknown their weights indicated that they were respectively about two months and twelve months. The latter age represents about one third of the life span of the rat. Both groups were treated identically with regard to food and quantity of germs fed. The virulence of the culture was tested on July 20 and 22, 10^7

TABLE I

PROTECTION AGAINST INTRAPEITONEAL INJECTION OF PNEUMOCOCCUS, TYPE I, AFFORDED RATS
BY FEEDING THE HCL ACID KILLED GERM C = CONTROL, E = TREATED,
D = DIED -DAYS, S = SURVIVED

DATE 1926	RAT NO	WT GM	DOSE C C	RESULT	DATE 1926	RAT NO	WT GM	DOSE C C	RESULT
3/21	C	180	10 ⁻⁵	D1	4/15	C	221	10 ⁻⁵	D2
	C	175	10 ⁻⁵	D2		C	196	10 ⁻⁶	D2
	343E	185	2x10 ⁻³	D2		C	180	10 ⁻⁷	D2
	329E	165	10 ⁻⁴	D2		C	171	10 ⁻⁸	D3
3/30	C	185	10 ⁻⁴	S	315E	210	10 ⁻³	D1	
	C	180	10 ⁻⁵	S		321E	215	10 ⁻³	D1
	342E	167	10 ⁻⁴	S		344E	230	10 ⁻³	D2
	351E	165	10 ⁻⁵	S		352E	230	10 ⁻³	D2
4/2	C	205	2x10 ⁻⁴	D2	327E	205	10 ⁻⁴	D2	
	314E	205	2x10 ⁻⁴	S		325E	206	10 ⁻⁴	D2
						337E	200	10 ⁻⁴	D2
						347E	195	10 ⁻⁴	D2
4/3	C	240	10 ⁻⁴	D2	4/17	C	255	10 ⁻⁵	D2
	C	203	10 ⁻⁵	D2		C	185	10 ⁻⁶	D2
	C	185	10 ⁻⁶	D2		C	170	10 ⁻⁷	D2
	313E	180	10 ⁻³	D2		C	167	10 ⁻⁸	S
4/5	310E	213	10 ⁻³	S	C	163	10 ⁻⁹	S	
	C	217	10 ⁻⁴	D2		317E	255	10 ⁻³	D2
	C	188	10 ⁻⁵	D2		316E	239	10 ⁻⁴	D5
	C	210	10 ⁻⁶	D2		320E	233	10 ⁻⁴	S
4/7	349E	220	10 ⁻³	D1	333E	205	10 ⁻⁴	S	
	335E	190	10 ⁻³	D2		331E	221	10 ⁻⁴	D2
	318E	195	10 ⁻³	S		324E	206	10 ⁻⁵	S
	A305E	207	10 ⁻³	S		A307E	233	10 ⁻⁵	S
4/9	C	222	2x10 ⁻⁴	D2	328E	200	10 ⁻⁵	S	
	C	220	2x10 ⁻⁵	D2		346E	190	10 ⁻⁵	D3
	C	203	2x10 ⁻⁶	D2		309E	195	10 ⁻⁶	S
	324E	240	2x10 ⁻²	D1		340E	175	10 ⁻⁶	D2
4/12	A303E	220	2x10 ⁻³	D1	5/5	C	182	10 ⁻⁵	D5
	319E	240	2x10 ⁻³	D1		C	165	10 ⁻⁶	D5
	323E	210	2x10 ⁻³	D2		C	143	10 ⁻⁷	D4
	338E	230	2x10 ⁻⁴	D1		C	143	10 ⁻⁸	D6
4/10	339E	224	2x10 ⁻⁴	S	373E	179	10 ⁻⁴	S	
	330E	228	2x10 ⁻⁴	D2		371E	173	10 ⁻⁵	S
	348E	208	2x10 ⁻⁶	S		369E	178	10 ⁻⁵	S
						374E	143	10 ⁻⁶	S
4/11	C	235	10 ⁻⁵	D2	5/6	C	150	2x10 ⁻⁵	D2
	C	203	10 ⁻⁶	D2		C	150	2x10 ⁻⁷	D3
	C	185	10 ⁻⁷	D3					
	C	174	10 ⁻⁸	S		C	195	10 ⁻⁵	D2
4/13	A308E	208	10 ⁻³	D2	5/10	C	166	10 ⁻⁶	D3
	311E	200	10 ⁻⁴	S		C	160	10 ⁻⁷	D2
	345E	225	10 ⁻⁴	S		C	155	10 ⁻⁸	S
	A306E	201	10 ⁻⁵	S		C	155	10 ⁻⁸	S
4/14	350E	197	10 ⁻⁵	S	370E	192	10 ⁻⁴	S	
	332E	180	10 ⁻⁵	S		356E	195	10 ⁻⁴	S
						355E	165	10 ⁻⁵	S
						376E	149	10 ⁻⁵	S
4/15	C	232	10 ⁻⁵	D2	5/14	C	220	10 ⁻⁵	D2
	C	202	10 ⁻⁶	D2		C	177	10 ⁻⁵	D3
	C	177	10 ⁻⁷	D2		C	185	10 ⁻⁶	S
	C	162	10 ⁻⁸	S		C	143	10 ⁻⁶	D4
4/16	326E	242	10 ⁻³	S	C	163	10 ⁻⁷	D3	
	336E	195	10 ⁻³	D2		C	143	10 ⁻⁷	D4
	A304E	160	10 ⁻⁴	D2		C	143	10 ⁻⁷	D5
	312E	175	10 ⁻⁴	S		C	160	10 ⁻⁸	S
4/17	334E	175	10 ⁻⁴	D2	C	140	10 ⁻⁸	S	
	341E	176	10 ⁻⁴	S		C	155	10 ⁻⁹	S

TABLE I—CONT'D

DATE 1926	PAT NO	WT GM	DOSE CC	RESULT	DATE 1926	PAT NO	WT GM	DOSE CC	RESULT
5/14	364E	173	10 ³	D4	7/20	C	131	10 ⁷	D3
	402E	166	10 ⁴	S		C	130	10 ⁸	D6
	363E	165	10 ⁴	S		375E	161	10 ⁴	S
	401E	141	10 ⁴	S		361E	159	10 ⁴	S
	354E	153	10 ⁴	D3		372E	142	10 ³	D4
	361E	144	10 ⁴	D2		C	125	10 ⁸	D6
	398E	190	10 ³	S		C	125	10 ⁷	S
	399E	170	10	S		C	125	10 ⁶	D3
5/19	C	223	10	D3	7/21	C	115	10	D2
	C	178	10 ⁶	D2		C	135	10 ⁶	D3
	C	148	10 ⁷	D3		C	285	10 ⁵	D2
	C	146	10 ⁸	S		C	310	10 ⁴	D2
	353E	178	10 ⁴	D6		C	335	2x10 ³	D4
	389E	170	10 ⁴	S	7/23	C	121	10 ⁶	D2
	390E	142	10	S		C	129	10 ³	D2
	350E	153	10 ⁵	S		C	252	10 ⁶	D2
5/21	C	160	10	D3		C	304	10 ⁵	D2
	C	158	10 ⁶	D3		419E	116	10 ⁶	S
	C	136	10 ⁷	D4		417E	124	10 ⁵	S
	C	135	10 ⁸	D4		445E	155	10 ⁶	S
	355E	183	1x10 ³	D4		450F	298	10 ⁵	S
	365E	146	10 ⁴	D4	7/26	C	117	10 ⁶	D2
	360E	138	10 ⁴	S		C	120	10 ³	D2
	400E	138	10 ⁵	S		C	295	10 ⁶	D3
5/24	C	200	10 ⁴	D4		C	305	10	D2
	C	173	10 ⁵	S		415E	110	10 ⁷	S
	C	148	10 ⁶	D6		407E	110	10 ⁵	D7
	C	146	10 ⁷	S		418E	320	10 ⁵	S
	C	141	10 ⁸	S		435E	325	10 ⁴	D2
	349E	175	1x10 ³	D3	7/27	C	133	10 ⁸	S
	368E	148	10 ⁴	S		C	138	10 ⁷	D2
	388E	147	10 ⁴	S		C	146	10 ⁶	D2
5/26	396E	145	10	S		C	153	10 ³	D2
	305E	147	10	S		C	312	10 ⁷	D2
	381E	144	10 ⁵	S		C	315	10 ⁶	D2
	C	225	2x10 ³	D4		C	318	10	D2
	C	145	2x10 ³	D4		427E	129	10 ⁵	S
	C	185	10 ⁴	D4		420E	131	10 ⁵	S
	C	150	10 ⁵	D4		411E	149	10 ⁴	S
	C	138	10 ⁶	D3		418E	151	10 ⁴	S
5/28	C	137	10	D3	7/30	431E	310	10 ⁵	S
	C	132	10 ⁸	S		429E	314	10 ⁵	S
	382E	153	10 ⁴	S		430E	322	10 ⁴	S
	366E	153	10 ⁴	D2		451E	323	10 ⁴	S
	394E	145	10 ⁴	S		C	154	10 ⁸	S
	378E	144	10 ⁷	S		C	156	10 ⁷	D2
	384E	143	10 ⁷	D6		C	158	10 ⁶	D2
	383E	142	10	S		C	165	10	D2
5/28	C	136	10	D2		C	300	10 ⁸	D2
	C	129	10 ⁶	D3		C	300	10 ⁷	D2
	C	128	10 ⁷	D2		C	305	10 ⁶	D2
	C	121	10 ⁸	D3		C	316	10 ⁵	D2
	385E	123	10 ⁴	S		414E	160	10	S
	E	126	10 ⁴	D3		410E	157	10 ⁵	S
	386E	121	10 ⁵	D2		421E	168	10 ⁴	S
	387E	119	10 ⁵	S		416E	171	10 ⁴	S
6/2	C	185	10 ⁷	D3		444E	300	10 ⁵	S
	C	138	10 ⁶	S		448E	301	10 ⁴	S

TABLE I—CONT'D

DATE 1926	RAT NO	WT GM	DOSE CC	RESULT	DATE 1926	RAT NO	WT GM	DOSE CC	RESULT
7/30	430E	308	10^{-4}	D2		C	156	10^{-7}	D4
	452E	312	2×10^{-3}	S		C	156	10^{-6}	D2
7/31						C	156	10^{-5}	D1
	C	153	10^{-8}	D2		C	273	10^{-10}	S
	C	154	10^{-7}	S		C	296	10^{-9}	S
	C	154	10^{-6}	D2		C	305	10^{-8}	D4
	C	157	10^{-5}	D2		C	311	10^{-7}	D6
	C	305	10^{-9}	D2		C	335	10^{-6}	D3
	C	303	10^{-8}	D3		C	335	10^{-5}	D2
	C	309	10^{-7}	D2		409E	170	10^{-5}	S
	C	320	10^{-6}	D2		403E	168	10^{-4}	S
	C	325	10^{-5}	D2		412E	171	10^{-4}	S
	405E	108	10^{-6}	S		416E	173	10^{-4}	S
	413E	145	10^{-5}	S		433E	270	10^{-6}	S
	425E	163	10^{-4}	S		437E	322	10^{-5}	S
	422E	169	2×10^{-3}	D2		446E	255	10^{-6}	S
	432E	298	10^{-5}	D2		443E	292	10^{-5}	S
	442E	311	10^{-4}	S		439E	323	10^{-5}	S
	449E	305	10^{-4}	D2		440E	323	10^{-4}	S
	447E	319	2×10^{-3}	D1		438E	325	10^{-4}	S
8/3	C	153	10^{-9}	S		441E	333	2×10^{-3}	D2
	C	153	10^{-8}	S					

c c killed a small rat and 10^5 c c a large one. Subsequently it was found, as the figures indicate, that a much smaller dose killed large control rats. On the 23rd of July, small and large controls died of 10^6 c c whereas the treated rats survived 10^5 c c. On the 26th, one small treated rat survived 10^{-6} c c, another died of this amount. It was the only rat of this group which failed to tolerate this quantity. Of two large rats tested, one survived 10^{-5} c c, the other succumbed to 10^{-4} c c. On the same date 10^6 c c killed both small and large controls. On the 27th, 10^7 c c killed both small and large control rats, whereas eight small and large rats fed the germs survived doses of 10^5 c c and 10^{-4} c c. On the 30th, a small control survived 10^8 c c, another died at 10^7 c c, whereas large controls died of both these amounts. Small experimental rats tolerated 10^{-4} c c and 10^{-5} c c. One large experimental lived after being injected with 10^5 c c, another after 10^{-4} c c, a third died of the latter quantity and a fourth survived 2×10^3 c c. This last animal therefore withstood at least 200,000 times a dose fatal for a control. On the 31st, a small experimental rat lived after receiving 10^{-4} c c but another died of 2×10^3 c c, 10^5 c c, 10^6 c c, and 10^8 c c, similarly killed small controls, a control injected with 10^7 c c survived. Of the large experimental rats three succumbed to 2×10^3 c c, 10^{-4} c c, and 10^{-5} c c, respectively, one receiving 10^{-4} c c survived. On this day 10^9 c c killed a large control. The unusual virulence of the culture explains the failure of a larger proportion of treated rats to survive. On August 3, 10^7 c c proved fatal for a small control, 10^{-5} c c for a large one. A large experimental rat died of 2×10^3 c c, eleven other rats receiving smaller doses all survived. There is thus consistent evidence showing that feeding the HCl acid-killed pneumococci protects rats whether young or adult, against subsequent intraperitoneal injection of the same living organism. The data show clearly that the adult rats are protected by this

treatment fully as well as the young ones. It is perhaps, therefore, not unsafe to assume that rats older than twelve months are also susceptible to immunization by this process, if not to an equal degree, at least partially.

It is of interest to note the relative susceptibility of small and large untreated rats to intraperitoneal injection of the pneumococcus. On July 23 and 26 both adult and young animals succumbed to 10^6 c.c. No smaller quantities were employed. On the 27th, 10^7 c.c. was likewise fatal to both kinds, 10^8 was not used on a large animal but failed to kill a small one. On the 30th, 10^8 c.c. again failed to kill a young rat but did kill an adult animal. On the following day this dose proved fatal for both kinds. Also 10^9 c.c. killed a large control. We do not know whether a small rat would have died of this amount because it was not used. One small control did, however survive 10^7 c.c. On August 3 an adult control succumbed to 10^8 c.c., whereas a young one survived. Although these figures are too few to warrant drawing a conclusion, they indicate that adult rats approximately one year old are less resistant to Type I pneumococcus than rats about two months old. There appears to be a similar relation in regard to toxic chemicals.³

Three separate experiments were done on mice with irregular results. It is at present impossible to explain the irregularity in the response of mice to the feeding process. The quantities fed were larger than required if calculated on the basis of weight of animal using the rat as standard.

In addition to the considerable difference obtaining between rats and mice in the regularity with which they can be immunized by this procedure there is another worthy of mention. Among the several hundred rats which have been fed either living pneumococci or the tissues of animals killed by intraperitoneal injection of this organism only occasionally has one died and never has the organism been recovered from the heart's blood of the dead rat, although efforts were made to do so. In October of 1925 when rats were being immunized by feeding the infected tissue an experiment was started in which mice were similarly treated using mouse tissue. Of 31 such mice 30 succumbed within a period of two weeks. Of 30 control mice fed on normal tissues of healthy mice sacrificed for the purpose 15 died in the same length of time. This shows that although mice fed raw tissue die mice fed on tissue containing pneumococci die in even larger numbers. Substantiation of these results was obtained in the experiments discussed above in which living germs alone were fed without tissue. Of 26 grown mice fed such organisms six died.

We have done only a few experiments with rabbits which yielded negative results.

It can now be stated that the extent of the immunity conferred by using acid killed organisms is as great as when pneumonic tissue or living germs are employed and that adult rats are as fully protected as young ones. It has previously been shown that the minimum duration of the protection afforded by feeding pneumonic tissue is four months⁴ at which time it is as marked as at the beginning. To a lesser extent it may last considerably longer. We do not yet know whether an equal duration of protection is procured when dead pneumococci are used. The use of acid killed organisms however, simplifies

any possible application of the procedure to human beings, which is the ultimate aim of the work. The similarities existing between the rat and man encourage the belief that the latter may also be immunized by feeding the pneumococcus.

Our present experiments are being made with monkeys.

CONCLUSIONS

1 Rats can consistently be immunized against intraperitoneal injection of pneumococcus, Type I, by feeding the acid-killed organism.

2 Adult rats are as well protected by this procedure as young ones.

REFERENCES

- ¹Ross, Victor Jour Immunol, 1926, **vi**, 219-229
- ²Ross, Victor Jour Immunol, 1926, **vi**, 237-249
- ³Donaldson, H H The Rat, Baltimore, 1924, ed 2, p 328
- ⁴Ross, Victor Jour Immunol, 1926, **vi**, 323-330

THE CHEMOTHERAPY OF STREPTOCOCCEMIA*

By ROBERT A. KILDUFFE, A.M., M.D., ATLANTIC CITY, N. J.

IN RECENT years a great deal of study has been directed toward the development of an efficient method for the treatment of blood stream infections by the intravenous administration of various dye compounds of varying degrees of bacteriostatic and bactericidal activity, among the more prominent of which is mercurochrome-220 soluble, a product formed by the combination of mercury and a fluorescent dye.

The literature abounds in conflicting reports as to the efficiency of this product. While, on the one hand, startling and dramatic instances are reported suggesting marked and rapid sterilization of the blood stream by this drug, on the other hand, equally significant reports are available in direct conflict with such conclusions.

Walker,¹ for example, in an investigation of the bactericidal properties of freshly defibrinated blood to which mercurochrome was added in varying concentrations, found no increase in bactericidal activity toward the colon bacillus in concentrations of mercurochrome as high as 1:400, with concentrations of 1:200 the bactericidal activity of the blood was destroyed. He also found that in mercurochrome-blood mixtures of 1:400, staphylococci and streptococci were not only not destroyed but that these organisms grew more luxuriantly than in plain blood. The explanation of this, according to Walker, lies in the injurious action of mercurochrome on the leucocytes.

The occurrence of numerous and directly contradictory reports of both clinical and experimental investigations has left the subject in a somewhat

*Read before the monthly meeting of the Atlantic City Hospital Staff, Friday, November 19, 1926.

Received for publication Nov. 27, 1926.

confused position and indicates the necessity for the collection and analysis of detailed and minute data of both kinds

It is apparent that clinical experimentation can seldom be conceived regulated, or followed out in strict accordance with preconceived plans as can be done in laboratory experiments, the various features of which are under more or less rigid control

It is possible, however, by the careful record of clinical minutiae to accumulate data capable of relatively accurate evaluation, and it may be contended that one case in which all the minutiae possible are painstakingly recorded may ultimately prove more informative than a series the account of which permits of *post hoc ergo propter hoc* conclusions

The present report is an endeavor to furnish such data

Until their enforced modification by the information thus far accumulated, two conceptions have dominated the chemotherapy of infection the conception of a *therapia sterilisans magna*, and that of a special chemical affinity of the drug and the organism

There is clinical evidence to support the former hope and experimental evidence to contradict and some, at least, of the opposed experiences are explainable on the basis of uncontrolled observations or unsupported assumptions and, particularly, as Churchman points out by a lack of definition as to what constitutes a bacteriemia

Certain facts seem assured

- 1 Under certain conditions and for certain bacteria, mercurochrome may be bactericidal and still under certain conditions such bactericidal property may be exhibited in the blood stream

- 2 For still other organisms mercurochrome may be bacteriostatic capable of inhibiting their growth without necessarily encompassing their destruction

- 3 Chemotherapy, as Kolmer has said, cannot be dissociated from the possibility of chemopathology which may be cumulative, dangerous, and even fatal

While Young, Scott and Hill³ have reported the ingestion of 900 mg of mercurochrome daily for ten days without injury and Hill and Bidgood⁴ that, unless the dose is at least 10 mg per kilo, renal damage does not follow the intravenous administration of mercurochrome, St George reports definite autopsy evidence of mercury poisoning in five mercurochrome treated cases, mercury being recovered from the viscera in amounts larger than those seen in chloride poisoning The largest amount given was 0.9 gm the smallest 0.1 gm

Corper⁵ likewise has called attention to the pathologic alteration of tissues directly exposed to the action of mercurochrome

It is apparent, therefore, that this, and comparable preparations suggested as chemotherapeutic agents, cannot be expected to produce inevitably a *therapia sterilisans magna* and that they must be used with care and due regard for the cumulative and even dangerous chemopathology which may result

The belief may be expressed that the true evolution of mercurochrome intravenous therapy of bacteriemia has been made difficult by the predominant importance given to its *bactericidal* activity and that attempts to achieve a *therapia sterilisans magna* have overshadowed all other possibilities and dominated its clinical, as well as, to a somewhat lesser extent, its experimental use.

The relatively large and frequent doses of solutions seldom weaker than 1 per cent and, when the concentration was lower, the use of larger quantities to compensate for the lessened strength of the solution, is apparent from the cases reported which lend support to this belief.

Nearly always efforts have been focused upon the massive and complete sterilization of the blood as the main, if not the sole, issue.

It can be debated if this is always the most important—or even the most desirable—consummation to be sought, for, as Churchman² has pointed out, septicemia—bacteriemia—may include a variety of conditions, the gravity of which is influenced by a variety of factors.

Among these are (a) the virulence of the invading organisms, (b) the accessibility to treatment of their source, the focus of infection, (c) the resistance of the patient, and (d) the site and gravity of the secondary lesions in various tissues or organs resulting from bacterial localization.

In every bacteriemia the bacteria enter the blood stream from some initial focus and are removed from the blood stream by the defensive mechanism, namely, the concerted, sequential and more or less predominant action of various protective resources by mechanical filtration by the lymph glands, by the formation of various specific antibodies, through phagocytosis, and so on. In the end-result it is always the nature as well as the vigor and completeness of the patient's reaction to the infection which determines the result.

It is fallacious, as Churchman comments, to assume that in bacteriemia the bacteria travel round and round in the blood stream reproducing as they go, and that injected antibacterial substances travel with them. It is more correct, and more likely, that there is an ebb and flow, a rise and fall in the bacterial content of the blood in accordance with repeated influxes from the primary focus, as well as from secondarily established multiple foci, and the repeated attempts at cleansing of the blood stream by the defensive agencies.

Admitting that mercurochrome and other comparable preparations can be introduced into the circulation only in relatively small amounts and, in terms of the blood volume, in very low concentrations (1/10,000 or over), that they do not remain long in the blood as such, that they are soon removed, and that they can hardly remain in contact with the bacteria long enough to exert a continuous or even prolonged bactericidal effect in terms of the infection as a whole, it seems quite probable that their bactericidal properties may have been given undue importance in past considerations.

Practically all bacteria, moreover, upon destruction and disintegration liberate greater or lesser amounts of endotoxin. Consideration of the inherent possibilities of the sudden liberation of massive doses of endotoxin consequent upon a rapid and complete sterilization of a heavily infected blood stream warrants the assumption that this may not always be desirable.

That such sudden absorption of endotoxin occurs can confidently be assumed from the character of the reaction occurring when the bactericidal action of mercurochrome is marked reactions characterized by chills fever sweating and, in some cases the picture of shock.

In the case reported all these factors were taken into consideration, the *bacteriostatic* rather than the bactericidal action of mercurochrome was sought and an endeavor was made to determine the frequency and amount given by a study of the blood count as eliciting information not readily apparent from the clinical picture.

The report of the case is made possible by the courtesy of Drs Theodore Sensemen and James H. Mason.

CASE I.—C. G. aged forty truck driver on the night of March 13 1926 while working at the rear of his truck, was struck by an automobile. He was admitted to the hospital suffering from shock and complaining of pain and loss of function in the left leg and thigh. Examination revealed a compound comminuted fracture of the middle third of the left femur, a small puncture wound on the posterior aspect of the left thigh and multiple abrasions over the site of the fracture.

The x-ray report of March 14 reads: Oblique fracture of the left femur just above the condyle. Step fracture about three inches above the knee.

A second picture on March 15 showed a dishing of the femur the middle fragment being above the upper and lower fragments with marked comminution of all three.

The fracture was again reduced and the leg placed in a double inclined plane x-ray showing the reduction to be satisfactory. Some difficulty was experienced however in keeping the fragments in good position and on March 20 an open reduction was done and a Lane plate inserted. Two days later without chill or rigor there was a sudden rise of temperature to 101.5 followed by a fall the next day to 99. At this time the patient complained of much pain in the leg which did not cease.

On April 10 fifteen days after the operation there was a considerable amount of drainage from the operative wound. The drainage continued and the temperature gradually rose the patient complaining unceasingly of pain and gradually becoming drowsy.

On April 16, three weeks after operation the temperature suddenly rose to 103 accompanied by a chill. At this time there was a large amount of seropurulent drainage. The temperature during the next few days ranged from 99 to 102 and the patient was restless irritable and without appetite. On April 21 five weeks after the injury three weeks after operation and five days after what appears to have been the initial invasion (April 16) the temperature rose to 105. The pain in the leg was not now so marked the patient possibly being too toxic to complain as bitterly and the drainage was greatly reduced in amount.

On April 22nd the temperature was 105.106 and the patient delirious. The leucocyte count was 16,400 with 81 per cent of neutrophiles and a leucocytic index of 68. A blood culture on this date within twenty-four hours gave a marked turbidity in 200 c.c. of bouillon the growth being a pure culture of nonhemolytic streptococci.

I first saw the patient on April 23. The temperature ranged from 103 to 104 the patient was delirious pallid covered with a clammy sweat and in very poor condition. A blood culture showed in twenty-four hours eleven colonies per c.c. of nonhemolytic streptococci.

The patient was obviously in a very desperate state and the prognosis grave. The use of mercurochrome 2.0 soluble was decided upon but with a definite departure from the strength and frequency of dosage usually advocated based upon the belief that the attempt to sterilize the blood stream by chemotherapy constituted only a part of the story and that sudden massive sterilization was not *ipso facto* necessarily desirable and in this case definitely contraindicated.

In view of the massive infection present in this patient as shown by the heavy growth in the blood culture and in view of his deplorable condition it seemed obvious that any at

tempt at massive production of bacterial death and disintegration must lead inevitably to overwhelming the patient by the sudden liberation and absorption of a large dose of endotoxin

The object in view was to produce, if possible, a definite degree of *bacteriostasis* rather than *bacteriolysis*, and to produce the minimum of reaction. Accordingly 5 c.c. of 1 per cent mercurochrome was injected intravenously just after taking a blood culture.

Three hours later, at 5 P.M., the patient had a severe chill and the temperature rose to 103°. At 4 A.M., April 24, the temperature had fallen abruptly to 97°, the patient was sweating profusely, the pulse weak, and the general condition that of collapse, this sequence of events being undoubtedly due to the liberation and absorption of streptococcal endotoxin.

On entering the ward on the morning of April 24 the change in the general appearance of the patient was startling and dramatic, his mind was clear and he was engaged in reading the paper. His temperature at 9 A.M. was 99° but at 11 A.M. was rising. The blood culture taken the day before previous to the injection showed ten colonies per c.c. of non-hemolytic streptococci.

At 11:30 A.M. a blood culture was taken and 5 c.c. of 0.5 per cent mercurochrome was injected through the same needle, this weaker solution being chosen because of the severity of the reaction after the first injection. By midnight the temperature had risen to 105° accompanied by transient spells of delirium and numerous chills and at 6 A.M. on April 25 had fallen to 100° accompanied by profuse sweating.

A leucocyte count April 25 showed 15,150 with 97 per cent neutrophils and a leucocytic index of 32.5, on the 26th the white count was 11,250, neutrophils 84 per cent, and leucocytic index 5.

The drainage from the wound, meantime, was small in amount, seropurulent, and showed on culture non-hemolytic streptococci, *Staphylococcus aureus*, and bacilli of Friedlander type. In an endeavor to attack the focus from which the blood stream infection was occurring and recurring, the wound was frequently freely irrigated with the mercurochrome-acetone solution described by Scott and Hill, diluted half strength.

On April 27 the white count was 11,276 with 76 per cent neutrophils and a leucocytic index of 3.6. The culture taken before the injection April 25 showed no growth after four days' incubation.

The temperature range was from 99° to 101° and the patient was apparently successfully battling his infection.

On the 28th, while his mental condition was somewhat hazy, the general condition seemed good, the temperature at 8 A.M. being 99°, leucocytes 12,100, neutrophils 73 per cent and leucocytic index 3.

At 2 P.M., however, the temperature suddenly rose to 103°. An immediate blood count showed a leucocytosis of 12,650 but a neutrophilic increase to 83 per cent, the leucocytic index being 6.6.

An intravenous injection of 3.5 c.c. of 0.5 per cent mercurochrome was given at 2 P.M., a blood culture being first taken. Within a few hours the temperature rose to 104°, dropping to 97.5° at 5 A.M., and rising to 100° by 8 A.M. the next morning.

The blood culture of the 28th gave 2 colonies per c.c. of streptococci. The white count on the 29th was 12,250, neutrophils 69 per cent, leucocytic index 2.6. A blood culture on the 30th was sterile.

The general condition remained satisfactory for some time, there was free drainage from the wound and a Carrel-Dakin drip was inserted and on May 5 a culture from the wound showed only a few staphylococci.

The general progress of the case was now very satisfactory in every way and an uninterrupted convalescence was expected. On May 13, however, the patient had a chill at 5:30 A.M. and at 2 P.M. the temperature had risen to 103.2° and 3.5 c.c. of 0.5 per cent mercurochrome was injected intravenously after first taking a blood culture. There was no perceptible growth in this culture until after four days' incubation when streptococci were recovered in small numbers.

At 11 P.M., eight hours after the injection the temperature had fallen to 98 and the following day the blood count showed 10,450 white cells and 87 per cent neutrophiles with a leucocytic index of 7

On May 15 the count was 9,300 with 74 per cent neutrophiles and a leucocytic index of 2.9

The drainage from the wound was very slight by this time and the Dakin solution was replaced by a saline drip

An x ray at this time showed extensive erosion of the femur in the neighborhood of the plate the upper screw of which appeared to have pulled out. The lower portion of the upper fragment of the lower fracture showed an area of absorption suggesting the presence of a pocket of infection

On June 8, the condition of the wound and patient being good the plate was removed under gas anesthesia. No pockets of infection were found

The wound healed nicely, no further events took place to complicate convalescence and the patient was discharged on June 26. He is now at work with very good functional results

It is to be noted that the total volume of mercurochrome injected in this case was 17 cc the total mercurochrome content being 0.11 gm, all quantities being much below the amounts usually given in a single dose. The frequency of dosage, moreover, was not arbitrary, but determined by the leucocytic as well as the clinical picture as indicating the progress of the battle between the patient and his bacterial antagonists

It is obvious that the result was not entirely dependent upon nor even markedly influenced by the bactericidal activity of the mercurochrome and that its bacteriostatic effect was an important if not the main factor

There is little doubt that in view of the general and desperate condition of the patient when treatment was begun he would not have withstood the reaction consequent upon the administration of large or frequently repeated injections of mercurochrome, without taking into consideration the possibility of producing mercurial poisoning

The fact that the focus of infection from which invasion of the blood stream occurred was accessible and amenable to treatment was also of importance in the favorable outcome

The following conclusions are suggested from this experience

- 1 The treatment of bacteriemia by the intravenous injection of mercurochrome 220 soluble should not be directed solely toward the immediate production of a *therapia sterilans magna*

- 2 The bacteriostatic effect of the drug is of equal importance with its bactericidal activity

- 3 From these premises it follows that the strength and amount of solution injected are not to be arbitrarily selected on the basis of the degree of bactericidal effect it may be possible to produce but should be determined by the clinical and bacteriologic picture of the particular case

- 4 The frequency of dosage should be determined by the leucocytic index as indicating the progress of the battle between the patient and the invading bacteria

- 5 Massive and frequent intravenous doses of mercurochrome 220 soluble are not necessary as a routine method of treatment

6 The sudden absorption of massive doses of endotoxin as a result of sudden and massive bacterial destruction in the blood stream possesses elements of danger and should be avoided

7 The production of some degree of reaction is to be sought for because of the therapeutic value of protein shock

8 It is possible to sterilize the blood stream in the presence of streptococcemia by the injection of small amounts of mercurochrome at regular intervals, the sterilization probably being due to the opportunity afforded by the induced bacteriostasis for the mobilization of the resisting powers of the patient rather than to the bactericidal effect of the drug

9 Treatment of an accessible focus of infection is an important element and should not be neglected

10 In view of the above premises both the strength of the dose and the frequency of administration of mercurochrome solutions should be modified

REFERENCES

- ¹Walker, J E Effect of Mercurochrome 220 Soluble On The Germicidal Properties of Fresh Defibrinated Blood, Arch Path and Lab Med, February, 1926, 1, 199-203
- ²Churchman, J W Intravenous Use of Dyes, Jour Am Med Assn, Dec 12, 1925, lxxv, 1849
- ³Young, J W, Scott, W W, and Hill, J H Use of Mercurochrome By Mouth as a Urinary and Intestinal Antiseptic, Jour Urol, September, 1924, iii, 237
- ⁴Hill, J H, and Bidgood, C Y Effect of Intravenous Injection of Mercurochrome on the Kidneys, Bull Johns Hopkins Hosp, December, 1924, xli, 409
- ⁵St George, A V Treatment of Sepsis With Mercurochrome 220 Soluble Report on Necropsies In Five Cases, Jour Am Med Assn, Dec 26, 1925, lxxv, 2005
- ⁶Corper, J H Pathologic Changes in the Lung from the Use of Mercurochrome 220 Soluble, Jour Am Med Assn, July 25, 1925, lxxv, 256
- ⁷Scott, W W, and Hill, J H Presentation of a Pre Operative Skin Disinfectant, an Alcohol Acetone Aqueous Solution of Mercurochrome, Jour Urol, August, 1924, vi, 135

LABORATORY METHODS

THE DETERMINATION OF SUGAR IN NORMAL URINE*

BY MARK R. EVERETT, PH.D. AND MRS. M. O. HART, B.Sc. NORMAN, OKLA.

METABOLIC investigations have figured largely in the recent revival of the question of the nature of sugar in normal urine. Our original purpose was to investigate the nature of this material by isolation experiments. However, literature concerning the comparative reliability of the most commonly employed analytic methods was so meager that it seemed best to first study comparative analytic values and then bearing upon existing knowledge of the nature of these substances.

Limiting ourselves to methods which had already merited some attention and which could be used advantageously for a long series of determinations, we chose the methods of Benedict and Osterberg,¹ Fohn and Beiglund,² and Sumner.³ In each case the latest published modification was employed. We call attention to the fact that the Sumner method used by us is essentially different from that used by Greenwald, Samet and Gross.⁴

Greenwald, Samet and Gross⁴ compared the glucose equivalents of several pure sugars by the analytic methods under consideration but this is only one phase of the analytic merit of the same methods when applied to urinary analysis. Sumner³ compared one of his earlier methods with that of Benedict and Osterberg.¹ Together with the analysis of urine from one person by Greenwald, Gross and Samet,⁴ this is the only comparative data available for the methods investigated by us.

A review with bibliography on the nature of sugar in normal urine is given by Greenwald, Gross and Samet.⁴ Since that time Host⁵ and Lund and Wolf⁶ have presented additional data to show that this sugar is not glucose.

According to recent investigations^{6, 7, 10} sugar of normal urine must be regarded as a variable mixture of substances. The accurate determination of such mixtures is a difficult problem and there is danger in attempting to draw quantitative conclusions from analysis of a limited number of urines. Our data represents several hundred analyses on urine from fourteen healthy men and women, twenty to forty three years of age. We feel however, that due to the variable and unknown nature of these reducing substances, we are not justified in claiming more than a qualitative significance for our results. The same criticism applies to other published data. We have calculated our results only to the nearest per cent believing that greater accuracy is impossible.

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 1, 1916 and 1st 1916.
From the Department of Biochemistry and Pharmacology, University of Oklahoma, Norman.

EXPERIMENTAL

Since our work was entirely of a comparative nature, the analyses were made under strictly comparable conditions. The sugar standards were made by dilution of the same stock solution of anhydrous glucose, C P, and were preserved with saturated benzoic acid solution. After proper dilution these standards were compared by the Folin-Wu method and were found to be exactly equivalent.

In the preparation of Sumner's reagent 3, 5-dinitrosalicylic acid, C P, Eastman Kodak Co., was used. Boneblack was prepared from commercial boneblack according to the directions of Benedict and Osterberg.

Unless otherwise stated, the analyses were made as soon as possible on one and one-half to four-hour period urines, voided by persons who during the previous twenty-four hours had eaten no toasted food or other food now known to give rise to large amounts of "foreign carbohydrates" in the urine. In this way we hoped to obtain samples of urine having a normal composition. Whenever precipitates appeared (as in alkaline urines) they were carefully suspended before taking the analytic sample.

Checks obtained upon the data reported never varied more than 1 per cent, with an average of 0.5 per cent, for the Folin-Berglund and the Sumner methods and never more than 2.2 per cent, with an average of 1.0 per cent, for the Benedict-Osterberg method. In the latter method the color produced

TABLE I
COMPARATIVE VALUES ON NORMAL URINE

NO	METHOD 1 MG / c c	METHOD 2 MG / c c	METH 1/METH 2 PER CENT	REMARKS
	Folin Berglund	Benedict Osterberg		
24	0.523	0.572	109	18 hr Urine
25	0.617	0.673	109	" " "
1	0.559	0.815	146	
9	0.696	1.183	170	5½ hr Urine P _H 7.4
11	0.537	0.800	149	
			Average	
			137	
			Average of 1, 9, 11	
			155	
	Folin Berglund	Sumner		
27	0.729	0.766	105	
28	0.531	0.552	104	
29	0.328	0.350	107	
1	0.559	0.600	107	
9	0.696	0.743	107	5½ hr Urine P _H 7.4
11	0.537	0.556	104	
12	0.269	0.261	97	
13	0.635	0.592	93	
			Average	
			103	
			Average of 1, 9, 11	
			106	
	Sumner	Benedict Osterberg		
30	0.952	1.645	173	
1	0.600	0.815	136	
2	0.502	0.746	149	
9	0.743	1.183	159	5½ hr Urine P _H 7.4
11	0.536	0.800	144	
			Average	
			153	
			Average of 1, 9, 11	
			146	

by the same amount of reducing substance is less intense and harder to compare than in the first two methods. This fact may explain the greater divergence in duplicate analyses by the Benedict Osterberg method.

Analyses by the Benedict Osterberg and Sumner methods were made on filtrates from urine treated with boneblack. The urine samples were acid in all but one case (No. 9).

The Folin Berglund method and the Sumner method, employing boneblack, gave quite similar results: variations of 7 per cent or less being found (Table I). In the eight direct comparisons of these two methods on different urines an average of 3 per cent higher values for the Sumner method was obtained.

Difference in the analytic values might be caused by an adsorption of sugar by Lloyd's reagent or by boneblack. Sumner found lower values for urine treated with boneblack and attributed this effect partly to a loss of sugar by adsorption. He found the adsorption of glucose more marked in neutral than in acid solution. If Lloyd's reagent adsorbs no sugar and if loss of sugar is the only factor concerned we would expect consistently lower values by the Sumner method. Such errors if they do exist may be counterbalanced by increased color production from other sources.

We tried the effect of thirty noncarbohydrate constituents of normal urine upon the reagents used in these methods. The presence of aldehydes, dioxybenzenes, cystine or ammonium salts, large amounts of each of which gave colors similar to the color given by glucose, appeared to be the most likely source of error for the Sumner method. However, none of these substances gave colors when present in amounts likely to be found in normal urine. Ammonium salts in concentrations corresponding to those found in certain pathologic samples of urine gave an appreciable color. Of the substances mentioned, aldehydes were the only ones yielding an appreciable color with the Folin Wu reagents. On the other hand, uric acid, allantoin, creatine, and creatinine also give colors with the Folin Wu reagents, but these substances are removed by Lloyd's reagent. Moreover, Folin and Wu¹¹ state that their older reagent is unaffected by 0.05 per cent of uric acid, creatine, or creatinine in the analytic sample. They do not say whether this is true for the new reagent or whether the somewhat larger amounts of creatinine and uric acid at times encountered in urinalysis, are equally harmless. We found that creatinine and uric acid in the concentrations occasionally found in very concentrated urine were not sufficiently removed by Lloyd's reagent to prevent the appearance of a slight color. However, these are not valid objections to the Folin Berglund procedure as such urine can and should be diluted before the analysis is made. Concentrated mammalian urine requires the same treatment because of the larger amounts of allantoin present. The small amount of allantoin in human urine has no effect on the Folin Wu reagents. Sumner says his reagent is "almost completely specific for reducing sugars" and we agree that it is more specific than the Folin Wu reagents as far as interference by creatine, creatinine, uric acid, or allantoin is concerned. Our present knowledge permits the conclusion that both methods are sufficiently specific to give identical values.

A more probable cause of the different values might be an increased color production by substances which do not themselves give colors but which increase the color given by sugars. Sumner discovered such an effect for phenols with his earlier reagent. The same relations may apply to all sugar methods for other urinary constituents.

Finally the individual sugars of normal urine may have different glucose equivalents for the two methods. Greenwald, Samet and Gross⁴ have determined the glucose equivalents of several pure sugars for the two methods but they used the older Sumner reagent.⁵

In comparison with these two methods the Benedict-Osterberg method gave higher and more variable values. Analyses by Greenwald, Gross and Samet⁶ of urine from a single individual reveal the same tendency. We calculated the ratio of Benedict-Osterberg to Folin-Berglund values reported by them and found the following averages:

	BENEDICT-OSTERBERG FOLIN-BERGLUND	VARIATIONS
Carbohydrate rich diet	139.5 per cent	121 per cent to 155 per cent
Carbohydrate poor diet	139 per cent	121 per cent to 156 per cent
Fat poor diet	169 per cent	137 per cent to 220 per cent
Varied diet	148 per cent	116 per cent to 173 per cent
Glucose and fat, or protein diet	155 per cent	122 per cent to 212 per cent

We could find no definite relations between these ratios and the dietary variations. Urine voided after glucose meals gave sometimes greatly increased and sometimes greatly decreased ratios. No apparent significance could be found in the relative values given by the two Benedict-Osterberg methods. Benedict and Osterberg¹ claim excellent agreement for these methods, but one can find variations of 20 per cent in their own reported comparisons, and as much as 65 per cent in the analyses of Greenwald, Gross and Samet. These divergences are certainly greater than those found by us between the Folin-Berglund and Sumner methods.

The factors previously mentioned might cause the high Benedict-Osterberg values. We found that large amounts of aldehydes and dioxybenzenes gave an intense color in their procedure. However, when present in concentrations likely to be found in normal urine, these substances gave no color. The color of the alkaline picrate solution was also affected slightly by a variety of other substances. The presence of certain amino derivatives, ammonium salts, ethyl acetoacetate and sodium bicarbonate caused a fading of the color of the reagent itself. The fading effect was produced by a few of these substances when present in amounts likely to be found in normal urine. While one may attribute the comparatively greater variations of the Benedict-Osterberg values to these effects, it is necessary to search elsewhere for the cause of the higher values.

There is no doubt that all the sugars examined by Greenwald, Samet and Gross, except glucosamine have higher glucose equivalents for the Benedict-Osterberg method than for the Folin-Berglund method. It is also probable that, just as in the case of the older Sumner method, increases of color are produced in the Benedict-Osterberg method by substances which them-

selves yield little or no color. Until the cause of the higher Benedict Osterberg values is definitely determined glucose equivalents of sugars are of doubtful value in elucidating the nature of sugar in normal urine.

Because of their great divergence from values secured by the methods of Folin and Berglund and of Sumner, we consider Benedict Osterberg values less reliable and more difficult of interpretation in metabolic experiments. This divergence is an important matter in metabolic experiments where differences of sugar excretion are measured. Some of the conflicting results in such investigations, may be explained on this basis (cf Benedict and Osterberg¹).

The Benedict Osterberg method is the most difficult of the three methods being discussed and the Sumner method is the simplest and shortest. There is a greater proportional color range for the Sumner method than for the Folin Berglund method.

TABLE II
COMPARATIVE EFFECT OF NORITE AND BONEBLACK

NO	METHOD	BOVEBLACK NO / C C	NORITE NO / C C	PER CENT	P _H	REMARKS
4	(a) Acid Urine					
8	Sumner	0.446	0.415	93	4	
5	"	0.602	0.704	117	4.5	
5	Benedict Osterberg	0.535	0.538	101	4	
7	"	1.099	1.031	94	4	6 hr Urine
	(b) Alkaline Urine					
4	Sumner	0.508	0.568	112	9.5	
8	"	0.735	0.806	110	8.5	
5	Benedict Osterberg	0.549	0.559	102	9.5	
7	"	0.913	0.976	107	9	6 hr Urine

In the experiments recorded in Table II, the urine was made acid or alkaline in each case by the addition of not more than one drop of concentrated hydrochloric acid or of 55 per cent sodium hydroxide solution per 10 cc of urine, so that the dilution effect was always less than 0.5 per cent and the difference in the dilution of the same urine never more than 0.25 per cent. In acid urine the use of norite gave variable results for both Sumner and Benedict Osterberg methods, while in alkaline urine it led to higher values for both methods, especially for Sumner's method. The filtrates from alkaline urine were decidedly more yellow than other norite or boneblack filtrates. The P_H of the samples was purposely beyond that encountered in normal urine in order to get a magnified effect. In all other analyses we adhered to the use of boneblack as the more desirable procedure.

CHANGES IN URINE ON STANDING

It soon became apparent that occasional changes in sugar values occurred in urine which had been standing several hours.

As was to be expected the greatest changes were encountered with long period samples. Retention in the bladder and standing of part of the samples in flasks gave increased opportunities for chemical changes and bacterial action. What part bacterial action might play in these changes could best be determined by the study of changes in preserved urine. It was first

TABLE III

NO	TIME AFTER VOIDING	FOLIN BERGLUND MG /C C	CHANGE PER CENT	BENEDICT OSTERBERG MG /C C	CHANGE PER CENT	REMARKS
21	2 hours	0 439		0 648		18 hr Urine
	6 "	0 506	+15	0 704	+9	
22	2 hours	0 480		0 596		16 hr Urine
	6 "	0 404	-16	0 721	+21	
23	2 hours	0 295		0 393		12 hr Urine
	6 "	0 291	-1	0 342	-13	
				Sumner		
27	2 hours	0 729		0 766		
	4 5 "	0 721	-1	0 760	-1	
29	2 hours	0 328		0 350		
	4 5 "	0 327	0	0 350	0	
		Benedict Osterberg				
30	2 hours	1 645		0 952		
	3 "	1 613	-2	0 948	0	

necessary to find out whether the preservatives themselves had any effect upon the analytic values

Portions of the original urine were shaken vigorously with the preservatives. As soon as the chloroform and toluene had separated, analyses were made simultaneously on all samples. Toluene had the least effect upon the values obtained by the Folin-Berglund method (3 per cent lower values), thymol had slightly more effect (5 per cent lower values), while greater variations were encountered with chloroform. The lower values for toluene preserved urine were not due to the inclusion of this preservative in the analytic sample as shown by the Sumner values for the same samples. Values secured by the Sumner method appeared to be least affected, being practically identical for the original urine and all preserved samples. The Benedict Osterberg values were also very little affected except in the case of chloroform-preserved samples which showed variations similar to those encountered with the Folin-Berglund method. Toluene and thymol, rather than chloroform, are the preservatives to be recommended for this purpose.

From the experiments recorded in Table V it is evident that changes are not entirely prevented by preservatives. The first two series of analyses are especially interesting. Urine No 12 showed decreased values for unpreserved samples by both Folin-Berglund and the Sumner methods. Values for toluene-preserved samples showed approximately the same decrease with the Folin-Berglund method, but slightly increased values with the Sumner method. Urine No 13 showed no appreciable changes when unpreserved but showed increased values when preserved with toluene. There is no doubt that bacteria were present in both unpreserved samples. Bacterial growth was prevented in the toluene-preserved urines for forty-eight hours. There is some doubt as to the ability of toluene to preserve urine indefinitely.

It seems evident from our data that bacterial action can remove part of the "sugar" and that a simultaneous liberation of increased amounts of reducing substances may at times counteract these effects. Since changes occur in toluene-preserved urine it is not possible to postpone the analysis without securing inaccurate results. The best plan in metabolic experiments is to analyze samples immediately and to deduce twenty-four-hour values from analysis of a number of short period samples.

TABLE IV
EFFECT OF PRESERVATIVES

NO	METHOD	ORIGINAL MG/C.C	CHLOROFORM MG/C.C	PER CENT	THYMOL MG/C.C	PET CENT	TOLUENE MG/C.C	PET CENT	PH	REMARKS
12	Folin Berglund	0.269					0.273	101	6.9	
13	"	0.635					0.635	100	6.5	
1	"	0.559					0.542	97	6.7	
2	"	0.571		116	0.517	94	0.523	92	6.5	
9	"	0.696	0.661	90	0.672	97	0.645	93	7.4	5 1/2 hr Urine
11	"	0.537	0.650	121	0.510	95	0.533	99	6.8	
					Average	95	Average	97		
14	Benedict Osterberg	0.746	0.775	104	0.769	103	0.749	100	6.5	
1	"	0.815					0.803	99	6.7	
9	"	1.183	1.149	97	1.143	97	1.143	97	7.4	5 1/2 hr Urine
11	"	0.800	0.870	109	0.833	104				
			Average	103	Average	101	Average	99	6.8	
12	Sumner	0.261					0.258	99	6.9	
13	"	0.592					0.595	101	6.5	
14	"	0.502					0.510	102	6.5	
1	"	0.600	0.499	99	0.511	102	0.602	100	6.7	
0	"	0.743	0.741	100	0.741	100	0.738	99	7.4	5 1/2 hr Urine
11	"	0.556	0.559	101	0.556	100	0.556	100	6.8	
			Average	100	Average	101	Average	100		

TABLE V
CHANGES IN PRESERVED URINE

TIME AFTER VOIDING	FOLIN BERGLUND				SUMNER			
	ORIGINAL MG / C C	CHANGE %	TOLUENE MG / C C	CHANGE %	ORIGINAL MG / C C	CHANGE %	TOLUENE MG / C C	CHANGE %
Urine No 12, P _H 6.9								
1½ hour	0.269		0.273		0.261		0.258	
3½ hours	0.262	-3	0.262	-4	0.255	-1	0.256	-1
25½ hours	0.253	-6	0.250	-8	0.237	-9	0.265	+3
Unpreserved sample cloudy								
Urine No 13, P _H 6.5								
1 hour	0.635		0.635		0.592		0.595	
5½ hours	0.641	+1	0.647	+2	0.599	+1	0.610	+3
25½ hours	0.635	0	0.672	+4	0.588	-1	0.629	+6
Unpreserved sample cloudy								

TIME AFTER VOIDING	FOLIN BERGLUND		BENEDICT OSTERBERG		SUMNER	
	TOLUENE MG / C C	CHANGE %	TOLUENE MG / C C	CHANGE %	TOLUENE MG / C C	CHANGE %
Urine No 1, P _H 6.7						
1 hour	0.542		0.801		0.602	
24 hours	0.533	-2				
27 hours			0.995	+24	0.615	+2

COMPARISON OF VALUES ON ACID AND ALKALINE URINE

Our last experiments were directed toward answering the question Is the hydrogen-ion concentration of urine partly responsible for changes in the sugar content of urine upon standing? The urine was made acid or alkaline in the manner described previously. The P_H of the samples was purposely altered beyond the limits to be found in normal urine (especially in the case of alkaline urine) in order to magnify the results. The effect of P_H on the analytic method employed is given in Table VI.

TABLE VI

NO	METHOD	ORIGINAL MG / C C	ACID MG / C C	ALKALINE MG / C C	ALKALINE / ACID PER CENT	P _H	REMARKS
3	Folin						
	Berglund		0.792	0.808	102	4 and 9	
10	"		0.365	0.432	118	4 and 9	
11	"	0.537	0.506	0.540	107	4 and 9.5	
				Average	109		
5	Benedict						
	Osterberg		0.585	0.549	94	4 and 9.5	
7	"		1.099	0.913	83	4 and 9	6 hr urine
				Average	88½		
4	Sumner		0.446	0.508	114	4 and 9.5	
8	"		0.602	0.735	122	4.5 and 8.5	
11	"	0.556	0.526	0.562	107	4 and 9.5	
				Average	114		

All analyses on urine No 11 were made at the same time. The filtrates in the Folin-Berglund procedure were always acid, P_H below 4 for the acid urines and about 6 to 6.5 for the alkaline urines.

The Folin-Berglund and Sumner methods gave higher values for alkaline urine than for the acid urine but, in the case of urine No 11, at least, the cause of this phenomenon was a lowering of the values for acid urine. The values for alkaline urine were practically identical with those secured

on the original urine which was slightly acid, (P_H 6.8) We may say, therefore, that an acid reaction has a tendency to give lower values with these two methods. The diminutions in acid urine are exactly the reverse of the expected results, if the sugar is adsorbed by boneblack in the same fashion as glucose (cf Sumner³). With the Benedict Osterberg method, we noticed exactly the opposite tendency, namely, lower values for alkaline urine. These results might be produced by differences in the adsorption of reducing substances by boneblack and Lloyd's reagent, or by variations in the reduction process itself. Of course exaggerated values were obtained by these experiments but it is very probable that similar, though smaller, deviations may be expected in normal urines where the P_H varies considerably.

The changes in sugar values produced by heating acid and alkaline urine are helpful in differentiating between the effect of the hydrogen ion concentration on the analytic procedure and on the "sugar" of urine itself (Table VII).

TABLE VII
EFFECTS PRODUCED BY HEATING FOR 10 MINUTES AT 100° C

NO	METHOD	BEFORE HEATING MO / C C	AFTER HEATING MO / C C	CHANGE %	P_H	TIME AFTER VOIDING	REMARKS
ACID URINE							
3	Fohn Berglund	0.792	0.833	+ 5	4	2 hours	
10	" "	0.365	0.400	+ 11	4	1½ hours	
11	" "	0.500	0.503	+ 11	4	3 hours	
			Average	+ 9			
5	Benedict						
	Osterberg	0.585	0.610	+ 4	4	¾ hour	
7	" "	1.099	1.105	+ 0.5	4	2½ hours	6 hr Urine
			Average	+ 2			
4	Sumner	0.446	0.467	+ 5	4	2 hours	
8	" "	0.602	0.606	+ 1	4.5	1½ hours	
11	" "	0.526	0.529	+ 1	4	3 hours	
			Average	+ 2			
ALKALINE URINE							
3	Fohn Berglund	0.808	0.816	+ 1	9	2 hours	
10	" "	0.432	0.430	0	9	1½ hours	
11	" "	0.540	0.393	- 27	9.5	3 hours	
			Average	- 9			
5	Benedict						
	Osterberg	0.549	0.365	- 34	9.5	¾ hours	
7	" "	0.913	0.840	- 8	9	2½ hours	6 hr Urine
11	" "	0.877	0.495	- 44	9.5	3 hours	
			Average	- 29			
4	Sumner	0.508	0.424	- 17	9.5	2 hours	
8	" "	0.735	0.714	- 3	9.5	1½ hours	
11	" "	0.562	0.424	- 25	9.5	3 hours	
			Average	- 15			

The urine was heated in volumetric flasks, cooled and made up to the original volume. All methods gave increased values with heated acid urines. The Fohn Berglund method was especially sensitive to this change which was exactly opposite to the effect produced by the mere presence of values given by the Fohn Berglund and Sumner methods. There marked tendency to decreased values with heated alkaline urine, with the Benedict Osterberg method.

TABLE VIII

	TIME AFTER VOIDING	ACID URINE MG / C C	CHANGE %	ALKALINE URINE MG / C C	CHANGE %
<i>Folin-Berglund Method</i>					
Urine No 3, P _H 4 and 9	2 hrs	0.792		0.808	
	45 hrs	0.792	0	0.808	0
	123 hrs	0.800	+ 1	0.792	- 2
Urine No 10, P _H 4 and 9	1 25 hrs	0.365		0.432	
	49 25 hrs	0.468	+ 28	0.441	+ 2
<i>Benedict Osterberg Method</i>					
Urine No 5 P _H 4 and 9.5	1 hr	0.585		0.549	
	93 hrs	0.749	+ 28	0.502	- 9
Urine No 7, P _H 4 and 9, 6 hr urine	2 5 hrs	1.099		0.913	
	56 hrs	1.130	+ 3	0.847	- 7
	128 75 hrs	0.741	- 33	0.633	- 31
<i>Sumner Method</i>					
Urine No 8, P _H 4.5 and 8.5	1 5 hrs	0.602		0.735	
	25 75 hrs	0.588	- 2	0.699	- 5

The object of the experiments recorded in Table VIII was to determine whether similar changes occurred in these samples upon standing. The samples were preserved with toluene.

The expected increases in acid urine were sometimes obtained. In one sample analyzed by the Benedict-Osterberg method there was later a marked decrease in sugar values. However, in samples which have stood as long as this one, the preservative value of toluene against microorganisms may be questioned. As a rule, the alkaline samples showed the expected decreased values. We are convinced that changes dependent upon P_H occur in preserved samples of urine and that the best procedure is to analyze the urine at once (at least within three to four hours after the sample is voided). Samples which have to be kept longer must be preserved and kept at a low temperature to avoid changes in apparent sugar content.

The variations encountered recall the fact that sugar of normal urine is a variable mixture of substances and no two urines may be expected to behave exactly alike. Increased values in acid solutions after standing may be attributed to hydrolysis of "combined sugars" (perhaps disaccharides). Decreased values in alkaline solutions may be caused by the destruction of sugar, or of some substance which increases the color produced by sugar. Similarly, the occasional decreased values in acid solutions may be attributed to the destruction of some noncarbohydrate substance.

The present analytic methods determine the reducing power of urine in terms of equivalent glucose reduction under specific conditions. Any substances which are not adsorbed by Lloyd's reagent from acid solution or by boneblack from acid or slightly alkaline solution, and which reduce the sugar reagents or increase the reduction of these reagents by sugar, are included in the term "sugar of normal urine."

CONCLUSIONS

The methods of Folin and Berglund and of Sumner give approximately the same values for sugar in normal urine while the Benedict-Osterberg method gives higher and probably less reliable values. There are several

factors which may be responsible for the differences in analytic values. Glucose equivalents for the urinary sugar may not be the same for the several analytic methods. There may be present in urine, substances which do not give colors themselves, but which increase or decrease the amount of color given by the sugar.

Somewhat irregular changes in the sugar content of normal urine take place after the sample is voided. These changes are of sufficient magnitude to affect the interpretation of published metabolic data. Preservation of urine with toluene does not entirely prevent such changes. Not all anti-septics are suitable for preserving the analytic samples.

The hydrogen ion concentration of the urine appears to affect both the analytic result and the nature of the changes which occur in voided urine.

REFERENCES

- ¹Benedict and Osterberg Jour Biol Chem, 1921 *lxviii* 51
- Folin and Berglund Jour Biol Chem 1922 *lx* 209
- ²Sumner Jour Biol Chem 1923 *lxv* 393
- ³Greenwald, Samet and Gross Jour Biol Chem 1924 *lxii* 597
- Sumner Jour Biol Chem 1924 *lxii* 247
- ⁴Greenwald, Gross and Samet Jour Biol Chem 1924 *lxii* 401
- ⁵Host Acta Med Scand Sup 1924 *vii* 180
- ⁶Luad and Wolf Biochem Jour 1925 *lxix* 538
- ⁷Folin and Berglund Jour Biol Chem 1922 *lx* 213
- ⁸Host Jour Metabol Research, 1923 *iv* 315
- ⁹Folin and Wu Jour Biol Chem 1919 *xxviii* 106
- ¹⁰Benedict and Osterberg Jour Biol Chem 1923 *lxv* 769

DISCUSSION

Dr Frederic E. Sondern—It is very evident that this communication is the result of a very extensive study. Dr Everett may be complimented on this paper.

Dr Wm Taylor Cummins—This is a subject in which we have been much interested in the Southern Pacific General Hospital, San Francisco. We have carried out the Sumner and Benedict colorimetric techniques for normal urine sugar coincidentally with the qualitative Benedict technique and have found in some instances that the latter gives figures twice as large as the former. The study of normal urine sugar was prompted sometime ago by the idea that some laboratory workers and clinicians were overestimating urine sugar in the qualitative Benedict technique. With this technique we place the tubes containing 3 c.c. of urine and 8 drops of Benedict solution in a beaker of boiling water for exactly five minutes. I wish to compliment Dr Everett on his work in which we are much interested.

Dr Mark B. Everett (closing)—In reply to Dr Cummins I was speaking of a qualitative test. I have some very definite replies for Dr Exton. He was speaking of an entirely different and older method which gave higher values. A new method has been devised and it is this method which I have been discussing. The values reported were secured on preserved samples of urine after four to twenty-four hours standing. I cannot recommend formalin as a preservative in sugar analysis. In regard to the question of the nature of these sugars, I have no doubt that sugar of normal urine is not glucose, but rather a mixture of reducing substances which are sugars and not other reducing substances as intimated by Dr Exton. I wish to emphasize that the disappearance from urine of these reducing sugars is appreciable within twenty-four hours. I am very grateful for Dr Exton's criticism.

A NEW MASK FOR USE IN BASAL METABOLISM DETERMINATIONS*

BY ALLAN WINTER ROWE, PH D, BOSTON, MASS

THE development of types of apparatus for the determination of the basal metabolic rate, which did not enclose the subject within a closed respiration chamber, has necessitated the elaboration of apparatus for connecting the subject with the gasometer. The joints thus made must be gas tight, as leakage is only less prejudicial to accuracy in the open than in the closed circuit method. The importance of the problem and the failure of complete success which has attended its solution is amply attested by the numerous devices described in the literature. Briefly stated, gas tight connection must be made through the mouth, the nose, or through both together. In event of but one orifice being utilized, the other must be hermetically sealed.

A standard method in rather general use is the rubber mouthpiece of Denayrouse¹ consisting of a rubber plate disposed between the lips and teeth, an outlet tube of ample dimensions passing between the former and two rubber offsets from the main plate which are engaged between the latter. Occasionally a case is encountered in which the ample dimensions of the mouth plate are inadequate. Loss of teeth rarely, if ever, proves a complication. The complement of this mouthpiece is a nose clip permitting exact closure of the nostrils. Several designs are available and all are reasonably effective. A certain percentage of cases encountered possess a nasal configuration which requires much pressure to effect a proper seal. This may be so strait as to be painful, in which event the absolute basality of the test is lost. On the whole, however, in my opinion, the mouthpiece and nose clip constitute the method of election because of their simplicity, ease of adjustment, cleanliness, sterility, and psychologic effect, this latter a factor that should never be ignored.

A second type of partial connection makes use of nosepieces. As originally designed by Tissot² these consist of glass tubes with a symmetrical circular enlargement, one of which is forced into each nostril. In the studies at the Carnegie Nutrition Laboratory,³ a first modification of these tubes involved the transformation of the terminal spheres to flattened ovoid form. Subsequently, small tubes were used with pneumatic shields made of thin rubber (dental dam) which were inflated after the tubes had been inserted in the nostrils. Mouth closure was effected by firm pressing of the lips, reinforced in those whose hirsute adornment would permit, with strips of adhesive plaster. Even in their last and blandest form the nosepieces, if gas tight, are definitely painful. Their use in a laboratory where scientific studies are being made on normals is feasible, but for clinical application and use

*From the Evans Memorial Boston, Mass

with the sick, they cannot be recommended. The discomfort entailed by their use destroys the basal state and leads to results which may be most misleading. Benedict and Benedict⁴ have called attention to the serious error that may be induced by minor body movements. In a study on the metabolism during pregnancy⁵ in which repeated observations were made on the same subject over considerable intervals of time the writer found that the slight movements associated with nervous tension and discomfort might introduce an error to the magnitude of over 20 per cent. This observation was unconsciously verified by Baer⁶ in a study on the same condition.

The use of a mask early suggested itself, and a variety of what may be termed "half masks" were utilized. Boothby and Sandiford⁷ use a rubber mask of rough triangular shape similar to that used for mine rescue work. This covers only the nose and mouth and is held in place by an elaborate network of tapes. A pneumatic rim which can be inflated they discard owing to change of tension in the peripheral pressure and possible leakage. Carpenter (1c) finds this mask unsatisfactory and substitutes one of sheet lead in the form of a cone, luted to the face with plasticene. Other commercial apparatus have made use of various modifications of the half mask attached to certain forms of anesthetic apparatus. With the half mask the mutations of contour of the human face make exact fitting a matter of much difficulty. The rubber masks require what at times is a painful pressure to render the joints gas tight, while the lead mask is patently ill adapted for clinical use.

The utilization of the full gas mask of warfare covering the entire face was first suggested by Bradley.⁸ Using a gas mask designed for use by the French Army, he adapts it to current purposes by adding pressure pads of five inch rubber sponges over the areas (temporal zones) where leaks are likely to occur. The apparatus is readily adjusted, gives a satisfactory closure and most important is comfortable.

Personal experience with the several types of apparatus here described led the writer to adopt the standard rubber mouthpiece and nose clip as most nearly conforming to the requirements of an active clinical service. As before stated, however, a small percentage of cases find it impossible to adapt themselves to the method. This group includes such types as the deep breathers with explosive expiration, mentally retarded adolescents with fluttering breath and the group where the anatomic configuration of the nose makes closure either impossible or disturbingly painful. To meet these contingencies a full mask seemed the most suitable device as I had duplicated Carpenter's experience with the rubber half mask. The war gas mask obviously possessed many advantages. Designed so to fit the face as to prevent leakage from the outside it remained only to modify it to arrest any complementary leakage from within. Inquiry developed the fact⁹ that samples of the perfected type of mask designed by Major Waldemar Kops for use with the A E F¹⁰ were available. This mask¹¹ was secured in three sizes, an important provision when the variation of size of head is considered. The mask fastens by a series

⁹I take pleasure in acknowledging my indebtedness to Bradley Dewey Esq. late Colonel, C.W.S. U.S.A. for most courteous and helpful assistance.

¹¹I take the greatest pleasure in acknowledging my basic indebtedness to Major Kops through whose generous gift the numerous masks used in this study were made available.

of elastic bands originating at the salient points of the periphery and uniting in a pad at the back of the head. The conventional slip buckle allows the independent regulation of the tension in each band and permits consequent accurate fit of the mask (see Fig 1). To secure closure against leakage from the inside, a thin-walled rubber tube, $1\frac{1}{4}$ " in diameter, was cemented along the inner proximal edge of the entire circumference. At the superior point a small rubber tube was introduced, terminating in the ordinary bicycle tire valve. Through this the tube could be inflated to any degree of tension by a small hand pump (see Fig 2).

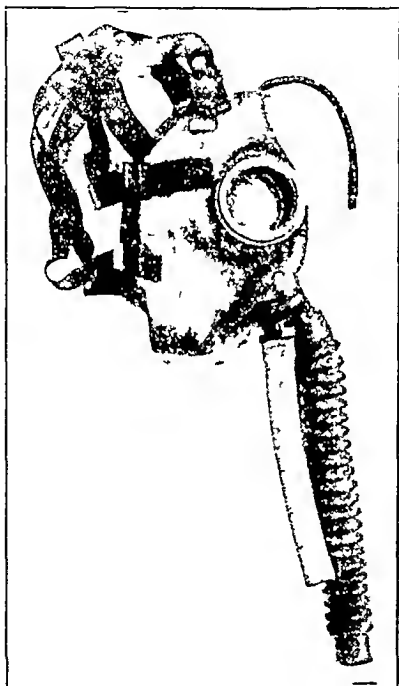


Fig 1



Fig 2

METHOD OF USE

The mask has been used with the Benedict-Collins type of closed circuit instrument⁹. As it has two outlets, however, it is equally adaptable to other types of apparatus, including all open circuit methods.

To apply the mask, it is adjusted to the head with the tube in the deflated state. Each elastic band is adjusted until the mask fits snugly and simple testing shows there is no appreciable inward leakage on inspiration. The patient next resting in a recumbent position (the mask is adjusted in a supported sitting posture), an interval is allowed to elapse to eliminate the effect of the earlier muscular effort. This also serves the purpose of familiarizing the subject with the mask, a not unnecessary precaution. The mask being comfortably and snugly adjusted, the hand pump is next attached to the

⁹For the time consuming experimentation necessary to prepare and fit this inner tube I am indebted to the co-operation of the Davol Rubber Co. Providence R. I.

tube and the latter inflated to the requisite tension. The pliable character of the tube and thus its easy adaptation to inequalities in the contour of the bearing surfaces renders it effective without discomfort. Soapsuds or grease may be used if there be reason to suspect leakage. Usually this has been found to be unnecessary. When the patient is resting comfortably the superior tube is connected with the apparatus and clamped off lightly while the latter is filled. During this part of the operation the patient breathes easily through the lower tube which is open. With the apparatus adjusted the clamp is removed and the lower tube closed by the rapid insertion of a



Fig 3



Fig 4

long tapered rubber stopper well greased and forced into the orifice to complete closure. Several minutes should now elapse—not less than four—to establish the gas equilibrium throughout the system. As the patient is in no discomfort and as additional oxygen may be introduced at will the element of haste is eliminated. At the expiration of the test disconnection is made and after deflation and loosening of the straps the mask is readily removed. In the writer's experience a heavy beard is the only inhibiting facial condition. Tests have been made successfully with subjects wearing small chin beards, as the bearing surface of the mask falls well toward the angle of the jaw (Figs 3 and 4).

The mask possesses certain advantages for purposes other than that for

which it was originally designed. As it causes no discomfort it can be worn for long periods of time without introducing the fatigue and nervous tension which seemingly cannot be dissociated from the other forms. By the exercise of certain obvious precautions it can be used in other than a recumbent position and is adapted to tests of metabolism in other than the basal state.

In conclusion, I wish to say that in routine clinical work, for reasons given above, I regard the standard mouthpiece and nose clip as the apparatus of election. The present device offers an efficient and satisfactory means of securing measurements on those patients to whom the routine procedure is ill adapted. Further, it can be applied to measurements made under a variety of physical conditions.

SUMMARY

A gas mask modified for use in basal and other metabolism determinations is described.

REFERENCES

- ¹Regnard. *Recherches experimentales sur les variations pathologiques des combustions respiratoires*, Paris, 1879, p. 286.
- ²Tissot. *Jour de physiol et de path gen*, 1904, vi, 688.
- ³Carpenter. *A Comparison of Methods for Determining the Respiratory Exchange in Man*, Carn Inst Pub, 1915, No. 216.
- ⁴Benedict and Benedict. *Proc Nat Acad Sci*, 1924, 498. *Bull Soc Sci Hyg Alim* xi, 1924, Nos. 8 and 9.
- ⁵Rowe, Alcott, and Mortimore. *Am Jour Physiol*, 1925, lxxi, 667.
- ⁶Baer. *Am Jour Obst and Gynec*, 1921, ii, 249.
- ⁷Boothby and Sandiford. *Laboratory Manual of the Technique of Basal Metabolic Rate Determinations*, Saunders, Philadelphia, 1920.
- ⁸Bailey. *JOUR LAB AND CLIN MED*, 1921, vi, 657; *Jour Biol Chem*, 1921, lxxv, 277.
- ⁹Benedict and Collins. *Boston Med and Surg Jour*, 1918, clxxviii, 567.

A NOTE ON THE METHOD OF DILUTING ANTIGENS FOR USE IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

BY ANNA C. MOORE, A. B., ALBANY, N. Y.

IN THE laboratory of the New York State Department of Health, two antigens are used routinely in the complement-fixation test for syphilis,—one, an acetone-insoluble antigen prepared by Bordet's¹ method and the other, a cholesterinized extract prepared by a method similar to that of Neymann and Gager.² The acetone-insoluble antigen is diluted by Bordet's method as follows. One part of antigen is evaporated to dryness. The dried residuum is then suspended in two and one-half parts of distilled water, the first cubic centimeter being added slowly with a 0.2 c.c. pipette and mixed as thoroughly as possible after the addition of each 0.2 c.c. The remainder of the water may be added rapidly, but the suspension must be thoroughly mixed. The appropriate amount of this suspension is then added to the amount of 0.85

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

Received for publication October 2, 1926.

per cent salt solution necessary to make the desired dilution which is mixed well by shaking. The cholesterinized antigen is diluted as follows. The appropriate amount of the cholesterinized extract is added rapidly to the amount of salt solution necessary to make the desired dilution and the dilution is mixed well by shaking. In the case of cholesterinized antigens, comparison is always made of dilutions prepared by both slow and rapid mixture of extract and salt solution and the method is adhered to which gives the more favorable results.

Recently, a portion of the acetone insoluble antigen was reinforced with 0.4 per cent of cholesterol for some experimental tests. When diluted in the same manner as the routine cholesterinized antigen and compared with it in complement fixation tests it gave much weaker results. Although it seemed improbable that antigens containing cholesterol could be satisfactorily suspended in salt solution after drying an attempt was made to dilute the antigen by Bordet's method in the same manner as the plain acetone insoluble antigen. There evidently was some precipitation of the cholesterol as the resulting suspension was somewhat grainy, but the dilution prepared by this method gave decidedly stronger results than did the dilution prepared by adding the fluid extract to the salt solution without evaporation.

It then occurred to us that certain cholesterinized extracts which had been found less sensitive than our routine cholesterinized extract might, by this method of dilution by evaporation have their antigenic properties sufficiently increased to be satisfactory for routine use. A cholesterinized extract (W 23) was selected which had previously been tested parallel with the routine cholesterinized antigen (W 17). Out of 195 complement fixation tests, the results had agreed in only 139. Of the 56 tests which had disagreed 52 had been stronger with the routine antigen the differences in about half these cases having been marked. This insensitive W 23 antigen was then diluted by Bordet's method of evaporation with gratifying results. Five hundred and twenty six comparative tests were made with it and the routine cholesterinized antigen and in 438 tests the results agreed. Of the 88 tests that disagreed 48 were slightly stronger with the routine antigen and 40 were slightly stronger with the W 23 antigen diluted by Bordet's method. In no case was the difference in the degree of fixation obtained marked. Thus Bordet's method of dilution renders this antigen satisfactory for use in routine complement fixation tests for syphilis whereas when diluted by the usual method it had proved too insensitive.

To determine whether the increased cloudiness of the suspension was the factor responsible for the increase in sensitivity a small series of comparative tests was made with a dilution prepared by Bordet's method and with a cloudy dilution prepared by adding salt solution slowly to the unevaporated extract. Approximately one fourth of the serums tested showed differences in the degree of fixation obtained with these two cloudy dilutions, the fixation being greater with the dilution prepared by Bordet's method in all the cases where differences appeared. The sensitivity of this antigen seems to depend to some extent therefore upon the method of its dispersion in salt solution.

Another cholesterinized extract, on the other hand, was found to give equally satisfactory results with cloudy solutions prepared by Bordet's method and by adding salt solution slowly to the unevaporated extract. Our routine cholesterinized extract gives equally satisfactory results with cloudy dilutions prepared by the two methods and with a clear dilution prepared by adding the extract rapidly to salt solution. The optimum dilution of the antigen is lower, however, when cloudy solutions are used, the 1:50 dilution being the optimum with cloudy solutions and the 1:100 dilution, the optimum with a clear solution.

These findings are at variance with the observations of Griffith and Scott,³ who state that the Bordet method of dilution applied to cholesterinized extract produces a suspension of diminished instead of improved efficacy. This conclusion might be reached if the titer of the antigen were taken as the measure of its effectiveness, since a rather anomalous fact noted by us was that there appeared to be no parallel between the titer of the antigen and its sensitivity. Antigen W 23 had a higher titer when diluted by adding the extract directly to the salt solution, but was decidedly more sensitive when diluted by Bordet's method of evaporation. When diluted directly, this antigen fixed completely in 0.1 c.c. of a 1:400 dilution and, when diluted by Bordet's method, it gave complete fixation with 0.1 c.c. in a dilution no higher than 1:100. The optimum dilution for the evaporated extract was found to be 1:50 and for the extract diluted directly, 1:100, the most sensitive results being obtained with this 1:50 dilution prepared by Bordet's method.

Other extracts which have been found too insensitive for use are to be tested by diluting the evaporated extract, and it is hoped and believed that these antigens will likewise be found satisfactory by this method.

A trial of this method for diluting antigens is recommended, since, with care in thoroughly suspending the dried residuum, a perfectly uniform dilution can always be made. On the other hand, when antigen is diluted by adding extract directly to salt solution or vice versa, there are bound to be slight variations, according to individual differences, in the degree of rapidity with which the mixture is made.

REFERENCES

- ¹Bordet, J., and Ruelens, G. L'antigène syphilitique de l'Institut Pasteur de Bruxelles, *Compt. rend. Soc. de biol.*, 1919, lxxxi, 880.
- ²Neymann, C. A., and Gager, L. T. A New Method for Making Wassermann Antigens from Normal Heart Tissue, *Jour. Immunol.*, 1917, ii, 573.
- ³Griffith, F., and Scott, W. M. *Technic of the Wassermann Reaction*, Ministry of Health Reports on Public Health and Medical Subjects, 1920 No. 1, p. 7.

A CASE OF POLYCYTHEMIA VERA TREATED WITH PHENYL HYDRAZIN HYDROCHLORIDE WITH SPECIAL REFERENCE TO CHANGES IN BLOOD MORPHOLOGY*

By HUGO O. ALTOW, M.D. AND JAMES B. CAREY, M.D. MINNEAPOLIS, MINN.

THE treatment of polycythemia vera by the use of arsenicals⁸ and benzol, by splenectomy,⁹ venesection and intravenous saline infusion,¹⁰ and even by irradiation in its various forms, has been so unsatisfactory that any new procedure is worthy of serious trial if there is any evidence at all that it might be successful.^{11, 1}

The hydrochloride of phenylhydrazin was first suggested and used by Eppinger¹ in the treatment of polycythemia vera following the demonstration by Moravitz and Pratt² that an anemia could be produced in animals by its administration. In his report of four cases Eppinger used the drug in 1 to 5 per cent solution by hypodermic injection subcutaneously in amounts varying from 2 to 12 c.c., beginning with small doses of the 1 per cent solution and gradually increasing both the strength of the solution and the size of the dose. Reduction in total red cell count and hemoglobin percentage, leucocytosis, jaundice and tenderness of the spleen were noted in all cases.

Tasehenburg³ followed up the work of Eppinger with essentially the same results. He reported in addition to a leucocytosis the presence of 15 per cent myelocytes and myeloblasts together with nucleated red cells when the total count had been reduced to 1,000,000 red cells and the hemoglobin percentage to 30. He used 9.65 gm. of the drug in divided dosage over a period of five and one half months.

The first reports in this country were by Trevor Owen in 1924⁴ and 1925. His use of the drug differed from that of the other authors in that he administered smaller doses, by mouth over an extended period of time.

None of these men apparently made any observations on the changes occurring in the blood cells themselves and it is on this account as well as to place on record another case of polycythemia vera treated with phenylhydrazin hydrochloride that we are submitting the following report.

Phenylhydrazin has a chemical formula of $C_6H_5-NH-NH$. The hydrochloride has been used in work on human subjects as being the least toxic form. The basic drug becomes oxidized to set free a benzol ring which probably is the actual active agent bringing about the blood destruction. It is a member of the antipyrim group of chemical substances and also, in its action, bears some relation to quinine.

The case we wish to report is that of a Jewish woman fifty three years of age married with husband and five children all living and well. Her

*From the Medical Division, The Nicollet Clinic, Minneapolis, Minn.
Received for publication, October 14, 1926.

family history is negative, and her past history uneventful except for an attack of pleurisy at the age of forty

During the summer of 1921 she passed tarry stools for a few days and in 1922 she had, apparently, a gastric hemorrhage. In November, 1923, she felt so ill that she sought medical advice at the Mayo Clinic

The blood examination at that time showed Hemoglobin, 81 per cent Red blood cell count, 5,570,000, hematocrit 56 per cent of red blood cells White blood cells, 5,100 Viscosity, 1.74 Whole blood volume, 151 cc per kilo

On the basis of these findings, and a palpable spleen, a diagnosis of polycythemia vera was made

The patient returned to Rochester in October, 1924, at which time the blood examination showed Hemoglobin, 84 Red blood cell count, 6,440,000 Viscosity, 1.76*

She presented herself at this Clinic on Nov. 27, 1924, complaining of vertigo, nausea, a sense of internal heat and generalized "neuralgic" pains. Our examination revealed a well-nourished woman, appearing somewhat younger than the stated age of fifty-three, with a high color, congestion of the conjunctiva and slight cyanosis of the mucous membranes. Physical examination revealed the liver edge one and one-half inches below the costal margin and the spleen three inches below the costal margin. The hemoglobin at this time was 89 and the red blood cell count was 6,970,000. From this time until November, 1925, she had extensive irradiation with the x-ray directed to the spleen and long bones, combined with benzol. The lowest blood count during this period was 5,100,000, and there was never at any time any symptomatic relief.

On December 31, 1925, she was hospitalized for treatment by phenylhydrazin hydrochloride. Her blood count on that date showed hemoglobin 95 (Dare), red blood cell count 7,340,000, white blood cell count 8,700. Subsequent blood counts during this treatment are charted (Fig. 1).

She received a total of 2.6 gm. of the drug in 0.1 gm. doses from December 31 to January 10, inclusive. During this time she became heavily jaundiced and the liver and spleen became enlarged and very tender. Her urine and stool contained bile and bile pigments in large amounts. The drug was discontinued on the tenth day of January, but the red cell count continued to drop until the lowest point was reached on the sixteenth. By that time the spleen and liver had receded, the jaundice had cleared up, and the excreta had returned to a normal content of bile and pigment.

On the eleventh day the fasting blood sugar was 0.128, the uric acid was 2.2, the urea nitrogen was 44.5, and the creatinine was 0.45. On this date the icterus index was 100+, according to the technique of Murphy.⁶

The patient was discharged from the hospital on the sixteenth and reported to the office on the twenty-third of January. At that time her hemoglobin was 74. The red blood cells had increased to 3,380,000 and the white cells to 6,100. She reported that she was much more comfortable than

*We are indebted to Dr. Giffin of the Mayo Clinic for these findings and for the privilege of reporting them.

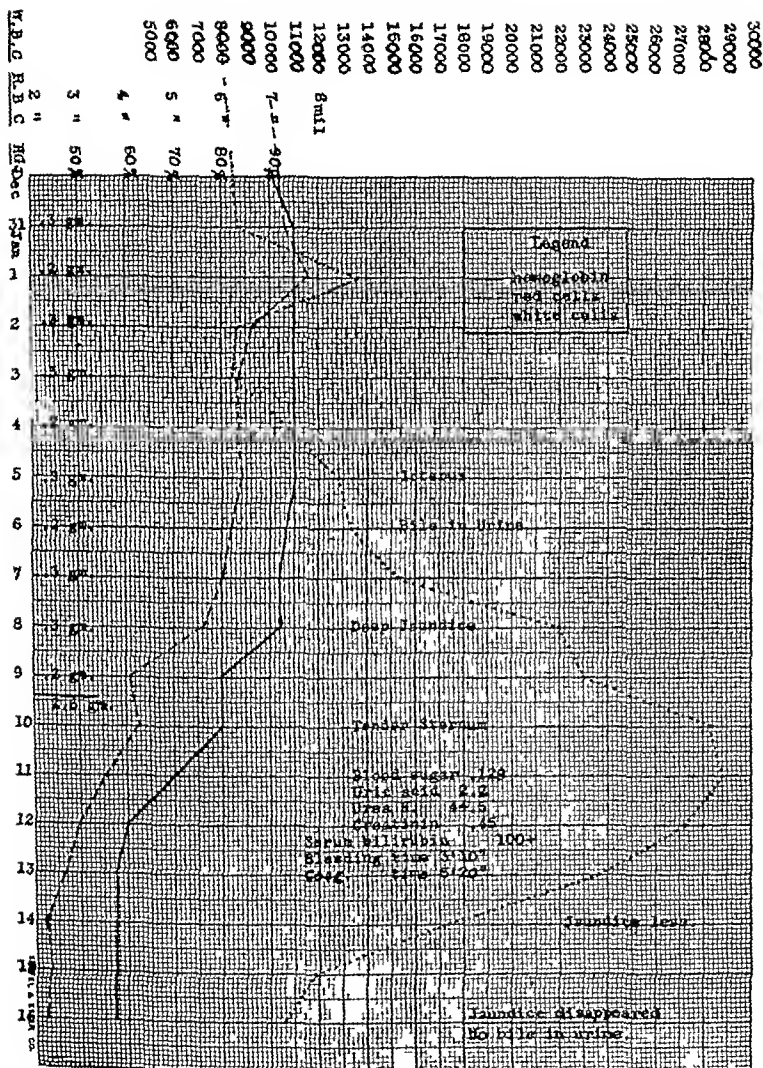


Fig 1

she had been for many years. The spleen was only about one and one half inches below the costal margin and the liver was not palpable.

A count on January 29 showed hemoglobin 78, red blood cells 3,680,000 and white blood cells 6,900. She felt so well that it was decided to undertake some dental work which she had long delayed. On Feb 19, 1926, she reported, after the dental work was completed, feeling very fine and with no complaints. The hemoglobin was then 80, the red blood cells 4,600,000, and the white blood cells 7,750.

On March 22 a blood count was made with the finding of hemoglobin 85, red blood cells 5,590,000. On April 10 the hemoglobin was 83, red blood cells 5,740,000 and white blood cells 9,500, and she was still free from complaints. Physical examination showed a palpable spleen, but otherwise normal findings.

On May 14 she reported at the office complaining of pain and "blisters" on the back and right side. She was found to have a herpes zoster on the right posterior thoracic region at about the level of the ninth vertebra. The blood findings at that time were hemoglobin 88, red blood cells 5,720,000 and white blood cells 6,700. It was planned that we would give her small doses of phenylhydrazin hydrochloride (probably 0.1 gm per day) as soon as the herpes had subsided.

It is evident from the results obtained in the above case that the drug is a powerful hemolyzing agent, and that the reduction in the total number of red blood cells has been produced by hemolytic destruction. The spleen and liver enlarged and became tender, but according to the figures obtained in the chemical study of the blood at the height of the hemolysis, there was no actual liver damage.

Levi¹ reports a case of polycythemia which had been treated with phenylhydrazin and which came to autopsy, in which a cirrhosis of the liver was found. This case had a total of 75 gm of the drug over a period of eighteen months and died of erysipelas. The autopsy showed a cirrhosis of the liver and enlarged spleen with infarction, coronary sclerosis, ulcers of the stomach and duodenum and a bronchopneumonia, but the history of the case showed that the man had been a heavy beer drinker, which might account in part for the hepatic condition. Also, cirrhosis of the liver has been reported many times as a finding at autopsy in polycythemia cases which had not been treated with phenylhydrazin.

In addition to the enlargement of the liver and spleen, hemolytic activity was further shown in our case by the deep jaundice of skin and sclerotics and the increased amounts of bile and bile pigments found in the excreta (urine and feces). Also, accompanying this very marked destruction of the red blood cells, or even preceding such destruction, there was an increase in the white blood cells, indicative of bone marrow stimulation. This was further predicated by the fact that the stool and urine were both freighted to capacity with bile and bile pigments, which could not occur to such a degree if the blood were only being destroyed, it being possible only because of a concomitant increase in production. There then followed, apparently, a depressant phase, perhaps of bone marrow exhaustion and depletion. Dur

During this period the total white count fell rapidly, the jaundice cleared up and the bile products disappeared from the urine and stool. At the same time our observation of the white blood cell picture indicated an effect on those elements which has not, in our survey of the literature, been reported in any other discussion of the subject.

TABLE I

Hg	R. B. C.	W. B. C.	LYMPHO CYTES	MONO CYTES	POLY S.	EOSINO PHILES	BASO PHILES	MYELO CYTES	OBSERVER
155	8,600,000	0,000	22.8	3.0	64.8	2.8	6		Weber
180	10,520,000	5,680	25.6	1.7	70.7	3	1.7		Weber
168	9,150,000	16,000	7.7	1.5	80.9	4.6	5.3	few	Von DeCastello
150-	8,000,000	6,600							
160	10,000,000	8,500	16.0	0	75.0	5.0	0	4.0	Kuttner
120	11,960,000	14,400	4.0	24.0	71.0	1.0	0		Umber
137-	7,900,000	5,300							
150	9,200,000	8,700	5.0	11.0	77.0	4.0	0		Beltz

In view of the fact that we did not interest ourselves in the intensive study of the whole blood until the thirteenth day after the beginning of the treatment, our data are incomplete. However the striking changes produced make it possible to interpret certain features in terms of action of the drug on the bone marrow and the reticuloendothelial system. Unfortunately no differential count was done before the administration of phenylhydrazin. An idea of the blood picture in erythremia in general may be obtained by examination of Table I which is a tabulation from E. Parkes Weber² of all complete blood counts given on patients with erythremia from the time that the diagnosis of this condition was first made up to the time of the publication of his monograph in 1921. It will be noted that the combined lymphocyte and monocyte count in only one case (that of Von DeCastello) approaches the low level reached in our case. We call attention to this feature to comment more fully on it later.

Furthermore it may be argued that in our patient permanent reduction in the lymphocyte and monocyte count may have been influenced by the previous irradiation and benzol treatment received before the administration of phenylhydrazin. Minot and Spurling³ have shown that the lymphocyte is the most sensitive cell to roentgen rays and that the lymphopenia is the last cell change to be adjusted. This is more particularly true when the irradiation has been sufficient to produce leucopenia. However in both instances, three days following irradiation there was a gradual increase in the lymphocyte count, so that at the end of approximately forty days the total lymphocyte count reached the level that existed before the exposure. In our patient a longer period since the last irradiation had elapsed.

Recognizing these objections and omissions in our data we shall proceed to analyze the blood counts of our patient as recorded in Fig. 1 and Table II. The most striking feature is probably that of the diminution of the red cell count, that, except for minor fluctuation was progressive and reached its maximum on the sixteenth day following the beginning of the administration of the drug. Clinical evidence supports the premise that this change was due to destruction of the red cells. Coincident with this and equally impres-

TABLE II

DATE	PLAT ELEMS	RED BLOOD CELLS						WHITE BLOOD CELLS					Remesles P A Number of Microcytes Large
		ANISO CYTOSIS	POIKILO CYTOSIS	POLY CHROMA SIA	ACHRO MIA	NU CLEATED REDS	RETICU LATED R B C	LYMPHO CYTES	MONONU CLEARS	POLYS	EOSINO PHILES	BASO PHILES	
1/12/26	N	++++	+++	+++	++	12		55	40	87	5	30	Basophile cells as found in Leuec mia Numerous broken r b c
1/13/26	N	++++	+++	++	+	0	84%	130	25	83.5	5	5	R B C picture still like that of Per nicious Anemia
1/16/26	N	++++	++++	++	+	0	47%	115	15	81.5	15	40	Many macrocytes and microcytes and pale red cells Nu merous "bizarre" lymphocytes
1/23/26	+1	++	+	+	++	0	24	70	30	87.5	0	25	Few microcytes and macrocytes
1/29/26	+2	++	+	0	++	0		55	10	92.0	10	10	Occas microcyte and poikloocyte Fairly normal r b c's
2/19/26	+2	++	++	0	++	0	4	90	10	87.5	5	20	Occas macrocytes
4/10/26	N	++	+	0	++	0		120	70	79.5	0	15	Less poikilocytosis Otherwise same
5/14/26	+2	++	+	0	++	0		90	65	83.0	20	5	

sive was the rising white blood cell count that reached its maximum from the tenth to the twelfth day. That this was due to marked stimulation of the marrow was made further evident by the presence of nucleated red cells in fairly large numbers on the twelfth day and 84 per cent reticulocytes on the following day. From this point there was a progressive decrease in the reticulocytes and on Jan 23, 1926, the count was 24 per cent, a figure which is slightly above normal. The smears taken on the twelfth, thirteenth and sixteenth days showed very marked anisocytosis with numerous macrocytes and microcytes (especially the latter) large pale red cells, fragmented cells and "ring" forms. In many respects the appearance of the smear was identical with that of pernicious anemia in a hemolytic crisis.

On the twenty third day there was a marked reduction in anisocytosis and poikilocytosis. This remained so on subsequent examinations and the last examination showed moderate anisocytosis, few poikilocytes and moderate achromia, on the whole more suggestive of simple chronic anemia. Platelet counts were not made, but the number of platelets was estimated, five to ten platelets per oil immersion field being considered as the normal standard. During the stage of active bone marrow activity when reticulocytes and white blood cells were increased, the platelets were apparently not increased. Later, when there was a decrease in these elements, there was a moderate increase in the number of platelets.

The effect of the drug on the white blood cell picture is more difficult to analyze. It is perhaps most easily comprehended if we consider separately the effect produced on the cells of the lymphocytic and myeloid series. It is generally accepted that the lymphocytes are produced in the lymph glands and the reticuloendothelial tissues of the spleen, liver, bone marrow, pharynx, and other reticuloendothelial structures. This is in conformity with the opinion of Weidenreich.²⁴ There is more uncertainty as to the origin of the monocyte or mononuclear cell, but the same author states that it can differentiate itself from these same structures. McJunkin² concludes that there are two types of mononuclear phagocytes present in human blood, namely, (1) monocytes, which are probably produced in the bone marrow and spleen, since they are found only in these fixed tissues, and (2) lymphendotheliocytes, arising from the lymphatic reticuloendothelium. Acting on these sources of information, it is reasonable to conclude that the source of production of cells of the lymphocytic series (lymphocytes and mononuclears) is in the reticuloendothelial system.

The statement that the polymorphonuclear leucocyte is chiefly produced in the bone marrow does not require substantiation, since this is the generally accepted view. These cells together with the polymorphonuclear eosinophile, constitute the myeloid cells of the peripheral blood. Weidenreich considers the bone marrow the usual site of origin of the eosinophile cells.⁴

The basophile cell must be considered as a special type of cell, neither lymphoid nor myeloid in origin. Weidenreich²⁴ (*ibid*, p 256), Pappenheim and Prosser (quoted by Weidenreich) consider the basophile or mast cell to be a degenerating form of the nongranular lymphocyte. This offers a satis-

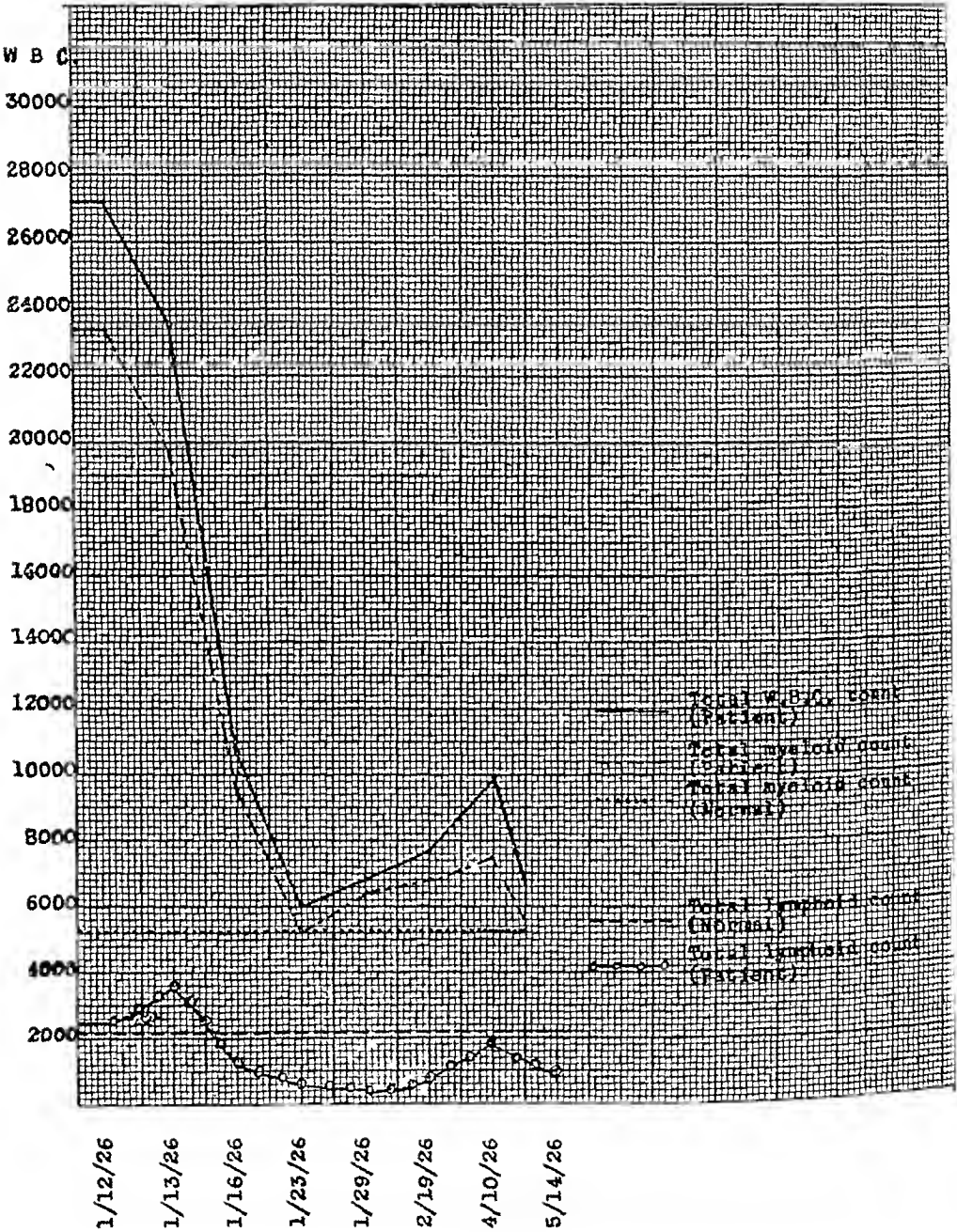


Fig 2

factory explanation for their appearance in large numbers in myelogenous leucemia where these elements are disappearing from the circulation.

We consider it necessary to establish the above facts as to the origin of the white blood cells before proceeding with our comment on the action of phenylhydrazin on the white blood cell picture of the subject of our case report. This action is portrayed in the behavior of the total absolute count of the cells of the myeloid and lymphoid series as shown in Fig. 2.

The curve of the myeloid cells (polymorphonuclear neutrophils and eosinophiles) parallels very closely the total white blood count. At no time does it fall below 5300 (total polymorphonuclear neutrophile and eosinophile count in normal blood on the basis of white blood cell count of 7500). The total lymphoid count (lymphocytes and mononuclears) apparently does not share in the initial increase as do the myeloid cells and shows approximately a 60 per cent increase as compared with an approximate 700 per cent increase in the latter on the thirteenth day. There is also a correspondingly greater decrease that is sustained for a longer period. At its maximum (January 29) it is less than 20 per cent of the normal total lymphoid count and at no time after the initial rise does it attain the normal figure (2100 on the basis of 7500 white blood cells in a normal individual). Judging from this it appears that the phenylhydrazin exerts a more depressant effect on the reticuloendothelial structure than it does on the other hemopoietic tissues. It is even doubtful as to whether it has any such action on the latter. On the other hand, it seems to be one of stimulation and the red blood cell count is driven down, not because of diminished red blood cell formation but because destruction is proportionately much greater than production. That the drug should exhibit a depressant or toxic effect on the reticuloendothelial system is not remarkable when we take into consideration the fact that according to the newer knowledge, hemolysis and the formation of bilirubin take place at the same site.

The basophile count is from two to eight times above the normal in all but two of the eight counts. If we accept the hypothesis of Weidenreich that they are a degenerative type of the nongranular lymphocytes and a cell undergoing retrograde metamorphosis then presence in increased numbers is easily explained when, as in this case the nongranular cells were diminishing in number. As additional support to this hypothesis we may also refer to Gruner⁶ who states that 'The lymphocyte of the blood stream, for instance, may undergo mucoid degeneration under the influence of certain toxins (staphylo toxin, phnolysin) and constitute the mast cell of the blood stream'. Other substances mentioned by him producing increased numbers of mast cells are pyridin, hemialbumose, colchicine, tuberculin, milk and cancer extracts. It is therefore quite possible that phenylhydrazin brings about an increase in these cells in a similar manner.

CONCLUSIONS

1. It is possible with the proper dosage and administration of phenylhydrazin hydrochloride to bring about complete amelioration of symptoms of the disease.

2 The use of the drug reduces the total red blood cell content of the blood, and can even produce an anemia

3 The reduction in the red blood cell count is an actual hemolytic destruction of these elements of the blood, evidenced by clinical and morphologic studies

4 There is also apparent a considerable stimulation of the bone marrow, as seen in the increase in total white cell count and number of nucleated red cells and reticulocytes

5 Administration of the drug in this case produced a marked reduction of the absolute lymphoid count, either by destruction of these cells or by a depressive effect on the reticuloendothelial structures inhibiting their formation

NOTE The subject of this report has been under constant observation since May, 1926. She was symptom free until November, at which time she required a course of the drug (0.1 gm daily for two weeks), which was repeated in December. Now, in March, 1927, she is comfortable again.

REFERENCES

- ¹Eppinger and Kloss *Therap Monatsh*, 1918, *xxiii*, 322
- ²Moravitz and Pratt *Munchen Med Wehnschr*, 1908, *iv*, 35, 817
- ³Taschenburg *Deutsch Med Wehnschr*, 1921, *lvii*, 27, 774
- ⁴Owen *Bull Johns Hopkins Hosp*, 1924, *xxxv*, 258
- ⁵Owen *Jour Am Med Assn*, 1925, *lxxvi*, 26, 2027
- ⁶Murphy *Bost Med and Surg Jour*, 1926, *cxciv*, 297
- ⁷Levi *Ztschr f klin Med*, 1924, *c*, 6, 777
- ⁸Hofman *Dermat Ztschr*, 1924, *xli*, 348
- ⁹Sauer *Deutsch Med Wehnschr*, 1924, *l*, 1635
- ¹⁰Betz Kaufman *Klin Wehnschr*, 1924, *iii*, 1841
- ¹¹Rydgaard *Hospitals Tidende (Copenhagen)*, 1921, *liiv*, 24, 379
- ¹²Bottan *Deutsch Med Wehnschr*, 1921, *lxv*, 27, 773
- ¹³Schnitze *Med klin*, 1923, *xix*, 458
- ¹⁴Mosenthal *Med Klin*, 1923, *xix*, 1158
- ¹⁵Gaisboch *Klin Wehnschr*, 1923, *ii*, 669
- ¹⁶Strashurger *Therap Halbmonatsch*, 1921, *xxxv*, 35, 777
- ¹⁷Pendergrass *Am Jour Med Sc*, 1921, *clxi*, 5, 723
- ¹⁸Wells *Jour Exper Med*, 1908, *x*, 457
- ¹⁹Underhill Kleiner *Jour Biol Chem*, 1908, *iv*, 165
- ²⁰Underhill *Jour Biol Chem*, 1914, *xvii*, 295
- ²¹Bodansky *Jour Biol Chem*, 1924, *lxviii*, 799
- ²²Weber, E Parkes *Polycythemia, Erythrocytosis and Erythrocytosis*, London, H K Lewis & Co, Ltd, 1921
- ²³Minot Spurling *Am Jour Med Sc*, 1924, *clxvii*, 2, 215-241
- ²⁴Weidenreich *Die Leucocyten und Verwandelte Zellformen*, Wiesbaden, J F Bergman, 1911
- ²⁵McJunkin *Arch Int Med*, 1925, *xxxvi*, 5, 799
- ²⁶Gruner *The Biology of the Blood Cells*, Wm Wood & Co, New York, 1914

A SIMPLE BLOOD CULTURE TECHNIC*

By PERRY J. MANHEIMS, M.D., AND IRENE S. McGRATH, A.B., NEW YORK CITY

THE use of an anticoagulant in taking blood cultures is not new, and various methods have been devised of simplifying the complicated procedures which are in more or less common use. Epstein¹ suggested the use of ammonium oxalate to prevent coagulation so that elaborate media at the bedside could be dispensed with. Chantemesse² and later Garbat³ in their work on blood cultures in typhoid fever, substituted sodium citrate for sodium oxalate. Ryttenberg⁴ used an ammonium oxalate and sodium chloride solution in taking blood cultures while Lintz employed a 1 per cent sodium citrate in normal saline solution or a 0.2 per cent ammonium oxalate in 0.6 per cent saline solution. Reichard⁵ advocates powdered sodium citrate as an anticoagulant †

The following technique has been employed in this laboratory for more than two years because of its simplicity and efficiency. One hundred c.c. of a 2 per cent sodium citrate solution is sterilized and kept as a stock solution. When a blood culture is to be made, 1½ c.c. of this is pipetted into a sterile cotton stoppered test tube (size 6x1) and boiled for a few minutes over an open Bunsen flame thus insuring sterilization of the citrate solution and at the same time making doubly certain of the sterilization of the tube. Approximately 1 c.c. of citrate solution is left in the tube which is then allowed to cool. It is only necessary to take to the bedside the test tube containing the sterile sodium citrate solution and a package in which are a sterile 10 c.c. Luer syringe and 2 needles (18-20 gauge).

The patient's arm is prepared in the manner usual for blood culture. 9 c.c. of blood are withdrawn and well mixed with the 1 c.c. of citrate solution in the tube, thus making a 0.2 per cent sodium citrate solution in blood, a percentage which is sufficient to prevent coagulation and which at the same time does not inhibit bacterial growth. The citrated blood is then taken to the laboratory and cultures made on suitable media. This is done as soon as possible after the blood is withdrawn but an hour's delay has apparently not interfered with bacterial growth.

For approximately six months the citrate method was paralleled with the old method of taking the media to the bedside. During this time there were 61 negative and 10 positive blood cultures and in every instance the results were the same with both methods. Since the citrate method has been adopted as routine our results have been as follows:

*From the Achells Laboratory of the Lenox Hill Hospital, New York City.

Received for publication October 3, 1926.

†Since the inauguration of this method, Colebrook and Storer⁶ have shown that sodium citrate inhibits the bactericidal power of blood and that this is more marked in freshly shed blood than in blood serum.

Total number of Blood Cultures--	535
Negative Cultures -----	457
Positive Cultures -----	78
B Typhoid -----	17
Staph Albus -----	13
Staph Aureus -----	10
Strep Viridans -----	10
Strep Hemolyticus -----	8
Pneumococcus -----	7
Bacillus Coli -----	6
Nonhemolytic Strep -----	5
Diphtheroid B -----	1
Meningococcus -----	1

The advantages of the citrate method of taking blood cultures are

- 1 This method is much simpler than those previously employed in which the media was carried to the bedside. It eliminates the difficulty of keeping the melted agar at the proper temperature while the blood is being taken.
- 2 The results are as good if not better than with the older complicated methods.
- 3 There is less chance of contamination because the cultures are made without haste and with proper facilities in the laboratory and not at the bedside.
- 4 There is less psychic upset to emotional patients.

CONCLUSIONS

The addition of 2 per cent sterile sodium citrate solution in sufficient quantity to prevent the coagulation of blood does not inhibit the growth of pathogenic organisms in this blood and provides a simple and satisfactory method of taking routine blood cultures.

REFERENCES

- ¹Epstein. Am Jour Med Sc New Series, September, 1907, cxxiv, No 3
- ²Chantemesse. Quoted by Ryttenberg
- ³Garbat, A. L. Personal Communication
- ⁴Ryttenberg, Chas. Jour Med Research, New Series, January, 1909, vi, No 1
- ⁵Lintz, Wm. New York Med Jour, April 3, 1915, ci No 14
- ⁶Reichard, J. D. The Military Surgeon, April, 1926, lvm, No 4
- ⁷Colebrook, L., and Stoier, E. J. Brit Jour Exper Path 1924, v, 47

STUDIES OF QUANTITATIVE BLOOD SUGAR ESTIMATION*

VARIOUS METHODS COMPARED WITH THE AUTHOR'S MICRO FOLIN WU METHOD

By THOMAS LUTHER BYRD M.D. MILWAUKEE WIS

DURING the past few years the chemical analysis of blood, for its various constituents, has gained an undisputed field in medical research. The data obtained by quantitative blood sugar estimations are not only of value, from a standpoint of diagnoses in cases presenting glycosuria and other symptoms complex, but furnish a better scientific knowledge as to the severity and the best rationale to observe in treating cases of both hyperglycemia and hypoglycemia, irrespective of the conditions causing these symptoms. As the standard methods of blood sugar determinations now in use require 2 cc or more of blood for a single analysis which necessitates a venipuncture, there are great numbers of patients including infants, small children and obese individuals, who cannot share in these advantages. Sugar tolerance tests are not done in many instances where they are indicated because of the obstacles encountered by both operator and patient in doing a series of venipunctures at frequent intervals.

One year ago I devised a means¹ requiring a minimum quantity of blood, applicable to all patients indicating the need of a quantitative blood sugar estimation. Comparative studies were made with the Folin Wu and the Micro Folin Wu methods² and with the latter method alone on a sufficient number of specimens of blood to demonstrate that the latter is just as reliable as the former method regardless of the sugar content of the specimens analyzed. These comparisons were made on a practical basis, that is the unknown of one technique was read as accurately as possible against its respective standard, the standards then changed and the unknown of the other technique brought to the same figure as the former and they invariably coincided.

Further comparative studies were made on fifty specimens of blood from both nondiabetic and diabetic patients with the Benedict modification of the Lewis Benedict, Myers Bailly, Folin Wu and the Micro Folin Wu methods. The three former methods were chosen because they are the standard methods commonly used as routine in the majority of clinical laboratories with slight modifications in some instances. The results are given in the accompanying tables.

TECHNIC

The original techniques were strictly adhered to in every detail. The standard of the Benedict modification of the Lewis Benedict method was pre-

*From the Laboratory of the Sacred Heart Sanitarium and St. Mary's Hill Milwaukee Wis.

Received for publication Oct 23 1926

TABLE I

COMPARATIVE ANALYSES OF BLOOD SPECIMENS FROM TWENTY FIVE NONDIABETIC PATIENTS
EXPRESSED IN MILLIGRAMS PER 100 CC OF BLOOD

NO	NAME	FOLIN WU METHOD USING 2 CC BL	MICRO FOLIN WU METHOD USING 0.1 CC BL	BENEDICT MOD LEWIS BENEDICT METHOD USING 2 CC BL	MYERS BALD METHOD USING 2 CC BL
1	Mrs F W G	095.2 mg	094.3 mg	108.1 mg	102.0 mg
2	Mrs A A	090.9 "	090.9 "	090.9 "	100.0 "
3	Miss A E	111.0 "	111.0 "	111.0 "	111.0 "
4	Mrs R E K	095.2 "	100.0 "	107.5 "	102.0 "
5	Mrs E W McD	100.0 "	100.0 "	100.0 "	100.0 "
6	Mrs J C	083.3 "	083.3 "	090.9 "	083.3 "
7	Mrs H O F	111.0 "	111.0 "	111.0 "	111.0 "
8	Mrs H G	092.5 "	090.2 "	098.3 "	095.2 "
9	Miss M E	100.0 "	100.0 "	120.0 "	111.0 "
10	Mrs E W K	087.6 "	085.7 "	111.0 "	097.5 "
11	Mr L O	103.6 "	103.6 "	115.3 "	099.2 "
12	Mr J O L	103.6 "	100.0 "	107.5 "	100.0 "
13	Mr M G	107.1 "	107.1 "	125.0 "	122.5 "
14	Mrs S M	100.0 "	100.0 "	111.0 "	111.0 "
15	Mrs N O L	084.5 "	083.9 "	097.5 "	101.9 "
16	Mr P S	097.5 "	097.5 "	098.4 "	093.2 "
17	Mr J P S	101.7 "	101.7 "	106.8 "	110.1 "
18	Mr J P	095.2 "	097.5 "	108.1 "	093.9 "
19	Miss O F	092.3 "	092.7 "	100.8 "	092.3 "
20	Mrs J A	092.3 "	095.2 "	101.7 "	096.8 "
21	Mr D F G	096.8 "	096.8 "	112.1 "	107.1 "
22	Miss H G	094.7 "	094.5 "	106.2 "	100.8 "
23	Mrs L M	101.7 "	101.7 "	104.3 "	103.5 "
24	Mr A T O	090.9 "	090.9 "	100.0 "	100.0 "
25	Mr J J C	097.5 "	098.3 "	101.7 "	099.2 "

A venipuncture was indicated in each instance for other blood tests and enough extra blood was obtained for sugar estimations and 0.1 cc was collected from the finger tip at the same time with the blood diluting pipette for the Micro-Folin-Wu method.

pared by dissolving 0.64 mg of pure anhydrous dextrose in 4 cc of a saturated solution of benzoic acid, instead of using a picramic acid solution for a standard, which is customary in most laboratories employing this technique. All specimens of blood were drawn after a twelve-hour fasting, by a venipuncture (potassium oxalate used as an anticoagulant), and from a pin prick in the finger, with the blood-diluting pipette for the micromethod. The standards and filtrates from each respective technique were boiled simultaneously in the same water-bath, the Folin-Wu tubes were removed after six minutes, and the other tubes allowed to boil seventeen minutes, the techniques terminated in the usual way. A standard (Eimer and Amend) biologic colorimeter was used, the cups measuring 15 cc with 2 cm diameter, the latter being of more importance than the capacity in regards to small quantities. The standards were set at the No. 10 on the colorimeter in each instance because of the small amount (6.25 cc) of the micromethod. Three independent readings were made on each unknown, added together, divided by three, the means taken as the figure to compute the values. For example, three readings of an unknown are 67, 7, and 69, added = 206, $- 3 = 686$, the means. The standard reading 10×200 (strong Folin-Wu standard) = 2,000, $- 686 = 291.5$ mg of sugar per 100 cc of blood. This will explain the odd figures in the tabulated results.

TABLE II

COMPARATIVE ANALYSES OF TWENTY FIVE BLOOD SPECIMENS COLLECTED FROM DIABETIC PATIENTS A NUMBER UNDER INSULIN TREATMENT EXPRESSED IN MILLIGRAMS PER 100 CC OF BLOOD

NO	NAME	FOLIN WU METHOD USING 2 CC BL	MICRO FOLIN WU METHOD USING 0.1 CC BL	BENEDICT MOD LEWIS BENEDICT METHOD USING 2 CC DL	MYERS BAILY METHOD USING 2 CC BL
1	Mrs H B	166.0 mg	166.5 mg	172.4 mg	179.0 mg
2	Mr A F (a)	201.0	201.6	214.3	203.4
3	" " (b)	171.5	172.7	172.1	171.5
4	" " (c)	400.2	487.8	387.0	363.8
5	" " (d)	666.6	606.0	500.0	428.5
6	Mr W W	155.0	155.0	157.9	168.1
7	Mr J C G	140.3	141.1	153.8	142.8
8	Miss E A	131.9	132.0	141.7	137.9
9	Mrs. D McG (a)	414.0	414.0	400.0	333.3
10	" " (b)	230.0	237.8	250.6	226.3
11	Mrs S R	223.8	223.2	236.4	240.8
12	Mrs. J J P	143.0	140.8	138.8	140.6
13	Mrs S S (a)	317.4	333.3	333.3	333.3
14	" " (b)	206.9	212.4	222.2	218.1
15	" " (c)	177.7	180.5	200.0	181.8
16	Mr F H (a)	153.8	153.8	166.6	160.6
17	" " (b)	141.2	147.2	146.4	135.3
18	Mr H F D	106.1	108.2	107.1	100.2
19	Mrs. C H G	301.4	301.4	322.5	312.5
20	Miss B C (a)	156.7	158.4	139.6	150.1
21	" " (b)	134.0	135.1	141.2	136.4
22	Mrs A. C (a)	292.8	291.5	297.6	291.5
23	" " (b)	270.4	277.7	280.8	295.2
24	Mrs K C	344.8	344.8	375.5	341.2
25	Mr H G	186.6	188.6	192.3	181.8

A venipuncture was done in each instance and 0.1 cc was obtained at the same time from the finger tip with the blood diluting pipette

METHOD OF PRESERVING HEMOLYZED BLOOD

Hemolyzed blood (1 volume of blood plus 7 volumes of distilled water) in any receptacle or in the blood diluting pipette as in the Folin Wu and the Micro Folin Wu methods, will maintain the sugar content nine to twelve hours at room temperature, which is sufficient where laboratory facilities are convenient, but not adequate when specimens are drawn at inconvenient hours for analyses or for mailing purposes. To overcome this difficulty it was necessary to find a preservative that could be added to distilled water, which would not interfere with hemolysis or alter the sugar content of the specimens to be analyzed. Several chemicals were tried and 1-400 formaldehyde solution (1 cc of commercial 40 per cent formaldehyde in 399 cc of distilled water) gave the desired medium. A number of oxalated specimens of blood were hemolyzed with this solution a portion analyzed immediately and other similar portions let stand at both room (75 to 85° F) and incubator (37.5° C) temperatures for periods of one, two, and three weeks respectively, and several samples hemolyzed in this manner in test tubes and the blood diluting pipettes were sent by mail to the Mayo Clinic, Rochester, Minn, (285 ml) and the University Hospital Augusta, Ga, (1,000 ml) without deterioration. The small differences in results are attributed to technical errors and minor changes in the sugar standards rather than the preservative being at

TABLE III

GIVEN TIME BLOOD SPECIMENS HEMOLYZED WITH 1.400 (40 PER CENT) FORMALDEHYDE SOLUTION, WILL REMAIN PRESERVED AT ROOM (75-85° F) AND INCUBATOR (37.5° C) TEMPERATURES EX IN MILLIGRAMS PER 100 CC OF BLOOD

BLOOD SPECIMENS	NO	1	2	3	4	
Folin Wu method, using 2 cc of bl	667			102	293	When taken
Micro Folin Wu method using 0.1 cc bl	667	411	105	292		When taken
Benedict Modification of Lewis Benedict Method, using 2 cc blood	500		100	298 303 286		When taken 2 wks at room temp 2 wks at incu temp
Myers Baily Method, using 2 cc of Blood	426			101 292 292		When taken 2 wks at room temp 2 wks at incu temp
Folin Wu Method, using 8 cc of hemolyzed blood, equivalent to 1 cc of whole blood, precipitated, filtered, yielding 2 to 5 cc of blood filtrate	667 667		408 420 412		103 102	1 wk at room temp 1 wk at incu temp When taken 2 wks at room temp 2 wks at incu temp 3 wks at room temp 3 wks at incu temp
Folin Wu Method, using 4 cc of hemolyzed blood equivalent to 0.5 cc of whole blood precipitated, centrifugated, yielding 2.5 to 3.5 cc of blood filtrate	667 667		408 427 420		102 102	1 wk at room temp 1 wk at incu temp When taken 2 wks at room temp 2 wks at incu temp 3 wks at room temp 3 wks at incu temp
Micro Folin Wu method, using 0.8 cc of hemolyzed blood equivalent to 0.1 cc of whole blood precipitated, centrifugated, yielding 0.5 to 0.7 cc of blood filtrate	667 667		411 426 432		101 102	1 wk at room temp 1 wk at incu temp When taken 2 wks at room temp 2 wks at incu temp 3 wks at room temp 3 wks at incu temp

These hemolyzed specimens were placed in unsterile 100 cc Erlenmeyer flasks and corked with rubber stoppers

TABLE IV

COMPARATIVE ANALYSES OF BLOOD SPECIMENS HEMOLYZED WITH 1.400 FORMALDEHYDE SOLUTION AND SENT TO OTHER LABORATORIES BY MAIL

BLOOD SPEC		FOLIN WU METHOD, USING 8 CC OF HEM BLOOD EQUIVALENT TO 1 CC OF WHOLE BLOOD	FOLIN WU METHOD, USING 4 CC OF HEM BLOOD EQUIVALENT TO 0.5 CC OF WHOLE BLOOD	MICRO FOLIN WU METHOD, USING 0.8 CC OF HEM BLOOD EQUIVALENT TO 0.1 CC OF WHOLE BLOOD	MICRO FOLIN WU METHOD, USING 0.1 CC OF BLOOD COLLECTED WITH BLOOD DIL. PIPETTE
DATE	NO 1				
1 28 25	When taken	276 mg	282 mg	282 mg	278 mg
1 31 25	Univ Hosp, Augusta, Ga (1,000 ml)	267 "	250 "	250 "	267 "
2 27 25	Mayo Clin, Rochester, Minnesota (285 ml)	288 "	288 "	288 "	293 "
2 26 25	No 2	214 "	215 "	213 "	214 "
3 5 25	Univ Hosp, Augusta, Ga (1,000 ml)	broken	broken	broken	214 "

Note—Specimen No 1 was analyzed at the Mayo Clinic one month after it was received

fault. There are four possibilities of a Folin Wu analysis of specimens preserved in this way, 2 cc of oxalated blood plus 14 cc of 1:400 formaldehyde solution furnish more than enough for three analyses, using 8 cc, 4 cc, and 0.8 cc of the hemolyzed blood, these being equivalent to 1 cc, 0.5 cc, and 0.1 cc of whole blood and precipitated accordingly. The fourth applies to 0.1 cc of blood collected with the blood diluting pipette using 1:400 formaldehyde solution as a diluent. Any of the above quantities of blood diluted properly (1 to 7 by volume) placed in a suitable container and sealed, or the pipette with a rubber band placed over the ends, can be sent any reasonable distance to a laboratory to be analyzed. Specimens for the Benedict modification of the Lewis Benedict and the Myers Bailey methods can be preserved likewise by diluting 2 cc of blood in proportion to each technique.

SUMMARY AND CONCLUSION

These comparisons were made with no intentions of placing discredit on any method used in this study but to give further evidence that the Micro Folin Wu method using 0.1 cc of blood is just as reliable as any method now in use irrespective of the quantity of blood necessary for respective techniques and regardless of the sugar content of the specimens analyzed. It can be applied to any case indicating the need of a quantitative blood sugar estimation including infants, small children and obese individuals. Its advantages are obvious.

A method of preserving hemolyzed specimens of blood giving four possibilities of a Folin Wu analysis making it feasible to collect and dilute specimens of blood with the blood diluting pipette and transport any distance in case laboratory facilities are not convenient.

REFERENCES

- (a) Byrd T. Luther. A Blood Diluting Pipette for Micro Folin Wu Method of Blood Sugar Estimation Using 0.1 cc of Blood. Preliminary Report Jour. Am. Med. Assn. Aug. 16, 1924, XXXIII, 508.
- (b) Byrd T. Luther. Pipeta Para Diluir La Sangre En El Micro metodo De Folin Wu Para El Calculo Del Azucar Sanguineo Utilizando 0.1 cc De Sangre. Informe Preliminar (Spanish) Jour. Am. Med. Assn. August 15, 1924, VII, 262.
- (a) Byrd T. L. A Micro Folin Wu Method of Quantitative Blood Sugar Estimation Using 0.1 cc of Blood. Wisconsin State Med. Jour. Feb. 15, 1925.
- (b) Byrd T. L. A Micro Folin Wu Method of Quantitative Blood Sugar Estimation Using 0.1 cc of Blood. Jour. of Lab. and Clin. Med. October 1925, XI, No. 1.

OXALIC ACID, A GOOD WHITE CELL DILUTING FLUID*

BY RODNEY JONES, DENVER, COLO.

ACETIC acid, the standard white-cell diluting fluid, while very good for a quantitative count, is not so good if a differentiation of the polymorphonuclear and the mononuclear cells is desired. It has too great a tendency to destroy the cell membrane and not leave a sharp differentiation between the nuclear and cytoplasmic substance.

After a series of experiments, in which the standard solution of acetic acid was compared with solutions of oxalic acid varying in strength from $\frac{1}{2}$ to 3 per cent, a 2 per cent solution of oxalic acid was found to be the best.

Oxalic acid in my experience comes much nearer being the ideal white cell diluting fluid. When used in a 2 per cent solution the red corpuscles are sufficiently destroyed, although not as completely as with the acetic acid. The white cells, due to the fact that neither the cell membrane nor the nucleus appear to be destroyed or distorted by the oxalic acid, stand out very clearly. Under low power a much more accurate determination of the percentage of polymorphonuclear cells as differentiated from the mononuclear cells can be made than when the acetic acid solution is used.

When the cells are examined under high power they appear very clear cut. The cell membrane appears as a light, rather homogenous, but nevertheless distinct shadow, while the nucleus is dark, regular, and in the case of the polymorphonuclear cells even the fine filament extending between the lobes of the nucleus can be demonstrated in the vast majority of cases.

*From the Department of Clinical Pathology University of Colorado School of Medicine,
Denver, Colorado
Received for publication October 2 1926

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D. ABSTRACT EDITOR

Clinical and Experimental

CEREBROSPINAL FLUID Value of Routine Examination of Cerebrospinal Fluid
Crawford, B. L. and Cantrow A. *Am Jour Med Sc*, June, 1926 *clxxi* No 6 p 859

Report of studies made in 210 cases

The total amount of spinal fluid is approximately 120 cc—completely replaced every five to six hours. Normally there is a constant balance between production and absorption. Acidosis, anoxemia, anesthesia, and the intravenous administration of extracts of choroid plexus, disintegrating brain tissue, and parietic fluids cause increased flow.

The colloidal benzoin reaction adds nothing of value to the routine examination.

To prevent disturbance of sugar content by glycolysis, specimens which cannot be examined at once should be placed in sterile tubes containing a few milligrams of sodium fluoride. They can be kept as long as a week at room temperature without marked change.

Normal variations of spinal fluid sugar occur with normal variations of blood sugar maintaining an approximate proportion of 1:2.

For protein content the authors use the following method: To 1 cc of spinal fluid add 7 cc of distilled water, 1 cc of 10 per cent sodium tungstate, and 1 cc of $\frac{1}{10}$ N sulphuric acid. Normal fluids give no precipitate.

In sixty-seven normal cases the cells were four or less per cu mm, globulin not increased, and sugar 40-60 mg per 100 cc determined by Foster's modification of the Folin-Wu technique and using a standard containing 5 mg glucose per 100 cc.

In eleven children the sugar content was from 71 to 90 mg with an average of 79 mg.

The essential cause of hyperglychorachia is increased permeability of the protective barrier of choroidal epithelium and cerebrospinal capillary endothelium, seen usually in conditions having an essential vascular pathology such as encephalitis and cerebrospinal syphilis.

Great increase in globulin and sugar content are characteristic of increased intracranial pressure. High sugar values were also found in various functional mental disorders.

The essential cause of low sugar values is glycolysis. It is marked in suppurative and slight in tuberculous meningitis.

The greatest value of sugar determinations is in the differentiation of tuberculous meningitis, from, especially, encephalitis epidemica. Sugar determinations should be a part of every routine examination.

PREGNANCY The Early Diagnosis of Pregnancy by Precision Further Observations of Sugar Tolerance Tests. Final Report Hirst J. C. 2nd and Long C. F. *Am Jour Med Sc*, June 1926 *clxxi* No 6, p 846

The following methods were reviewed:

1. The Abderhalden reaction concluded to be of no value because of the large number of nonspecific reactions.
2. Erede's anaphylactic reaction without experimental substantiation.
3. Casta's novocaine-formalin reaction: numerous nonspecific reactions with normal blood and in infections and toxic conditions.
4. Diemst's reaction: valueless even in pregnancy.
5. Sedimentation test: worthless for the diagnosis of pregnancy though of interest in other conditions.
6. The alimentary glycosuria test of Frank and Nothmann: A useful test with a margin of 6 to 8 per cent error.

The method used is as follows

A Average supper given the night before

B Collect first morning specimen of urine which must be negative to Fehling's solution

C Omit breakfast

D Give 75 gm of table sugar per 10 pounds of body weight except that the maximum amount must not be over 150 gm This is dissolved in two glasses of water each containing the juice of half a lemon

E Voided specimens are collected at one and two hours after the sugar ingestion and qualitatively tested for sugar by Fehling's method

If either of the hourly specimens shows a definite reaction for sugar the test is positive "Traces" or "slight reductions" are disregarded The reactions must be frankly positive or negative

Precautions must be taken to see that the entire dose of sugar is taken and that none is lost by vomiting Patients intolerant to sugar because of various conditions are not amenable to the test

7 The Raubitschek adienalim test inconstant and untrustworthy

8 The Phlorizin test prone to false positive results and untrustworthy

DIABETES Relation of Abdominal and Rectal Infections to the Pathogenesis of Diabetes Mellitus, Vishe, J W Am Jour Med Sc, June, 1926, clxvi, No 6, p 836

Case reports tending to support the following premises

The underlying pathologic change in diabetes in many cases is a pancreatitis

The pancreatitis may originate in acute infectious diseases, and from hematogenous focal infections

Five cases of diabetes mellitus apparently secondary to abdominal and rectal infections are reported, with improvement following surgical intervention

The opinion is ventured that in these cases the infection reached the pancreas through the lymphatics, either directly or by way of the portal circulation

The conclusion is suggested that abdominal and rectal infections are important etiologic factors in the etiology of diabetes mellitus

CARCINOMA The Bacterial Flora of Cancer of the Breast, Warren, S L. Am Jour Med Sc, June, 1926, clxvi, No 6, p 813

Micrococci and diphtheroids have been cultured readily from cancer tissue obtained from seven human breasts, without obvious areas of infection They also were present in a breast with chronic mastitis and in parts of a breast not involved by cancer

It would appear that these organisms are casual inhabitants of the breast structure, and play no direct part in the production of cancer of the breast

The literature bears witness to the fact that Nuzum's micrococcus, which seems the same as the one described here, has been obtained at different times in the past though given various names

CARCINOMA The Repeated Inoculations of Animals with So Called "Cancer Organisms," Warren, S L, and Pearse, H E Am Jour Med Sc, June, 1926, No 6, p 820

Two hundred and forty one mice of a strain susceptible to mouse cancer inoculations, but in which spontaneous tumors were very rare, were given at weekly intervals intracutaneous injections of either the micrococcus of Nuzum or diphtheroids and micrococci obtained from human breast cancers The inoculations were continued until death, or for four months, at which time only fifty mice remained alive, the others having died, usually from septicemia These fifty mice were observed for two months more, or for a total of six months

Ulcerations of the skin which readily healed occurred with great regularity None of the animals showed any evidence of a neoplastic growth except one which developed a spontaneous tumor of the liver

Four rabbits receiving weekly injections of both diphtheroids and micrococci for three to five months, showed no signs of malignant disease even at the end of six months

No evidence was found that any of these organisms play a primary role in the etiology of cancer though an indirect role is possible

PREGNANCY Interagglutination of Maternal and Fetal Blood in the Late Toxemias of Pregnancy, Allen W M Bull Johns Hopkins Hosp, March, 1926, XLVIII, No 3 p 17

A study concerned with the possible relation of blood incompatibility as a cause of eclampsia in which the reagglutination relationship of 479 mothers and their infants was investigated

Microscopic technic was used routinely

The cases were divided into six groups (1) normal (2) questionable, (3) preeclamptic toxemia, (4) eclampsia (5) nephritic toxemia without convulsions (6) nephritic toxemia with convulsions

There is no evidence that incompatibility is more frequent in toxemic than in normal gestation Incompatibility between the bloods of mother and infant was present in 20.8 per cent of 375 normal and 21.1 per cent of 104 toxemic pregnancies

There is no evidence of an increased agglutinin titer in the maternal serum of toxemic women

There is no evidence of specific immunization of the mother against fetal corpuscles

The discrepancy between this and previous work is probably accounted for by the size of the series studied With a small number of cases the percentage of error and likelihood of coincidence are very great

The study of this series of cases by the methods used, gives no evidence that the late toxemias of pregnancy have their origin in reagglutination phenomena

TUBERCULIN TESTS Intracutaneous Tests with Human and Bovine Tuberculin Downing H F and Higgins H L Am Jour Dis Child February 1926, XLVI, 1, 8

From a study of fifty one cases the following conclusions are formulated

Both bovine and human tuberculin should be employed in routine tuberculin tests not only to determine the diagnosis of the type of tubercle bacilli present but also to increase the value of the test by detecting cases which would otherwise be missed In our series the missed cases would have amounted to 15 per cent of the total tuberculous patients

Evidence from two cases tend to confirm earlier reports that in the first stages of tuberculosis, the patient will react to but one type of tuberculin presumably that of the infecting type while later the patient reacts to other types (group reaction)

UREMIA On the Presence of Cyanate in the Blood Gottlieb E Brit Biochem Jour 1926, XX, 1

In view of the fact that it has been asserted that considerable quantities of cyanate are found in the blood and, also, that cyanic acid may give rise to uremia Gottlieb reports his experiments on this matter

Cyanate is rare in the blood in concentrations over 0.1 mg per 100 cc of plasma

Cyanate per os or intravenously is toxic hence cyanate as present in ordinary solutions cannot be considered as a precursor of uremia

MERCUROCHROME Unsuccessful Experiments with Mercurochrome as a Biliary Antiseptic IX. Experimental Typhoid Paratyphoid Carriers Meyer K F Sommer H and Eddie B Jour Infect Dis, June, 1926, XLVIII, No 6 p 469

Mercurochrome intravenously injected is excreted in the hepatic bile of rabbits in concentrations which may destroy 10,000,000 typhoid bacilli in from six to twenty four hours It has been impossible, however to cure experimentally produced gall bladder carriers by giving mercurochrome intravenously or by mouth

The colorimetric methods used in the estimation of mercuriochrome, the influence of proteins, etc., on the germicidal properties of the compound are discussed

Laboratory Technique

BLOOD SEDIMENTATION The Graphic Presentation of the Blood Sedimentation Test. A Study in Pulmonary Tuberculosis, Cutler, J. *Am Jour Med Sc*, June, 1926, clxx, No 6, p 882

Reviewing the variety of methods which have been and are employed in this procedure and which render difficult an accurate comparison of results, Cutler presents a simple method and a graphic report utilized by him in a study of pulmonary tuberculosis

A 5 cc tube, graduated in tenths of a cubic millimeter, each 1 mm in height, and marked in millimeters is required. This tube may be obtained from the A. H. Thomas Co., Philadelphia, Pa.

Blood is aspirated to the 5 cc mark in a syringe containing 0.5 cc of 3 per cent sodium citrate (freshly made), mixed by tilting the syringe, and, after removal of the needle, emptied into the tube. Several specimens may be taken at once and the tubes marked.

On arrival in the laboratory, the tubes are stoppered with paraffined corks and gently inverted several times.

They are replaced in the rack and readings made every five minutes for one hour, the readings being graphed on a special chart. Readings may be made any time up to ten hours after collection of the blood. On these charts, obtainable from C. M. Beckmeyer, Sellersville, Pa., the horizontal lines represent the divisions of the sedimentation tube, the vertical lines intervals of time. A graph is thus constructed.

The author uses the term "sedimentation index" to express the total sedimentation of the blood cells at one hour.

The normal index for men is from 2 to 8, for healthy women from 2 to 10, during menstruation as high as 12 mm.

By "sedimentation time" is meant the period elapsing before packing of the red cells sets in. Normally this is always a question of hours.

The greater the sedimentation index and the shorter the sedimentation time, the greater is the pathologic activity and vice versa.

In healthy individuals the graph is always a straight line.

Cutler regards the test as a nonspecific reaction occurring in many diseases but also looks upon it as a valuable aid in estimating the activity of tuberculous lesions.

He emphasizes the value of the graphic method described.

The paper is illustrated with six figures including a reproduction of the chart.

BLOOD SUGAR A New Titrimetric Principle and Its Application to the Determination of Uric Acid and Blood Sugar, Flatow, L. *Munchen med Wehnschr*, November 20, 1925, lxxii, 2009

Proteins are removed with 10 per cent sodium tungstate and two thirds normal sulphuric acid.

The following reagents are required.

1 Potassium ferricyanide 0.0803 gm, distilled water 100 cc. Keep in a dark bottle. The solution is stable. 1 cc = 0.1 gm of dextrose.

2 Sodium indigomonosulphate 0.3 gm in 1000 cc of distilled water.

3 A 15 per cent cold neutralized sodium carbonate solution and its tenfold dilution.

Method 0.1 cc of blood is taken from the finger with a standardized pipette and transferred to a centrifuge tube containing 1.7 cc of distilled water. Add 0.1 cc of tungstate solution and mix, add 0.1 cc of sulphuric acid and shake well.

Centrifuge and transfer 1 cc to a wide test tube, (or 0.5 cc plus 0.5 cc of distilled water) add 2 cc of ferricyanide solution and 0.5 cc of 15 per cent sodium carbonate solution.

In a second tube, (blank test), place 1 cc of water, 2 cc of ferricyanide solution and 0.5 cc of 15 per cent sodium carbonate solution

Place both tubes in a water bath and boil fifteen minutes. Cool and titrate with the indigomonosulphate solution until the first bluish color is constant for 1 minute. A few drops of the 15 per cent sodium carbonate solution are added before the titration.

The amount of indigo required for titration in the sugar test (b), is subtracted from the amount used in the blank test, (a), and the result divided by (a) and multiplied by 0.2 equals the sugar content

$$\text{Sugar} = \left(\frac{a - b}{a} \right) \times 0.2$$

The method as applied to the determination of uric acid is indirect and cumbersome

IMMUNITY The Isolation of Substances with Immune Properties Locke, A. and Hirsch E. F. Jour Infect Dis November 1925 xxxii 449

A method is presented for the preparation of a highly purified hemolysin by selective adsorption and a destruction of the combining capacity of the homologous erythrocyte by ether extraction

The procedure permits the securing of hemolysin of such purity that but 0.000125 to 0.00018 mg of protein are associated with each hemolytic unit

Fresh sheep blood is defibrinated, centrifuged and the cell sediment washed five times with 0.9 per cent sodium chloride solution. Fifteen cc of the packed washed cell sediment are equally divided, between two large centrifuge tubes and to each portion there is added quickly and with vigorous shaking 40 cc of perfectly fresh rabbit antiserum. After two hours the cells have completely laked and the stroma has flocculated and settled toward the bottom of the tubes. Centrifugation for forty five minutes completes the separation, 90 to 99 per cent of the hemolysin originally present is found to be bound to the stroma sediment. The brilliantly clear, red supernatant liquid is decanted from the sediment and replaced by an equal volume of 0.9 per cent sodium chloride solution. After the stroma is finely suspended in the wash liquor by prolonged shaking, the suspension is allowed to stand for twenty minutes and is then strongly centrifuged for thirty minutes. The washing process is repeated (about six times) until the supernatant liquid has no trace of color and gives no trace of foam when shaken. The stroma obtained is perfectly white and has lost little of the originally bound hemolysin.

The well washed, hemolysin saturated stroma is extracted with ether three times. The volume of the stroma decreases 90 per cent under this treatment. After the removal of the third ether extract the ether remaining dissolved in the stroma material is removed by centrifugation in a warm centrifuge. The stroma material packs at the bottom of the tube and the salt solution, which made up the cell volume may be decanted. The residue is washed twice with 0.9 per cent salt solution and once with distilled water. Considerable hemolysis is lost to the salt solution but protein impurities due to surface adsorption, are thereby almost completely removed. The hemolysin of the salt extracts may be recovered by electro dialysis and isoelectric flocculation. The washed residue is extracted repeatedly with N/1000 sulphuric acid the extracts are pooled flocculated by neutralization, and the suspension centrifuged. The flocculation is quantitative as hemolysin is almost insoluble in pure water. The precipitated material is extracted with 0.9 per cent salt solution and solutions of any desired hemolytic content may be obtained by varying the amount of salt solution used. The hemolytic unit of the extract is associated with from 0.00015 to 0.00018 mg of protein. The preparations still contain a small amount of cell globulin but are probably 30 per cent or more pure hemolysin. Their combining power for erythrocytes far exceeds that of ordinary immune serums, indicating that their content of stroma material is very small. The amount of the original hemolysin which is recovered in these preparations is 30 to 50 per cent.

BLOOD, IRON IN Determination of Iron in Blood, Tissues, and Urine, Fowweather, F
S Brit Biochem Jour, 1926, 22, 93

Method of determination of iron in blood—One c.c. of blood is measured into a test tube containing 4 c.c. of distilled water. After thorough mixing, 1 c.c. of this diluted blood is transferred to a Pyrex test tube (200 x 25 mm) followed by 1 c.c. of concentrated sulphuric acid. The tube is clamped and held at an angle of 40° to the horizontal. The contents are boiled rather vigorously until practically all the water has been driven off and white fumes begin to be evolved. Heating is discontinued for about half a minute, after which time 0.5 c.c. of "perhydrol" is added to the tube, drop by drop, from a test pipette. Boiling is then repeated. A brisk evolution of oxygen occurs and the solution in the tube assumes an amber color. When white fumes are again evolved, heating is again discontinued and after cooling for half to one minute a further 0.5 c.c. of "perhydrol" is added as before. Heating is resumed and the solution usually becomes colorless. If all the color has not disappeared an additional 0.3 c.c. of "perhydrol" is added. Heating is continued for one minute after the solution has become colorless. The solution is now allowed to cool completely, then it is diluted with about 5 c.c. of distilled water and transferred to a 50 c.c. stoppered graduated flask. Into another similar flask are placed 1 c.c. of a standard iron solution containing 0.1 mg. iron per c.c. and 1 c.c. concentrated sulphuric acid. Water is added to both flasks up to a volume of about 18 c.c. after which 25 c.c. acetone are added. The contents of the flasks are thoroughly mixed and allowed to cool at room temperature. To each flask are then added 5 c.c. of 3 M ammonium thiocyanate, the contents mixed and made up to the mark. The two solutions are then compared in a colorimeter.

If the standard is set at 20 mm, then

$$\frac{20}{R} \times 50 = \text{mg iron per 100 c.c. blood,}$$

or $\frac{20 \times 50}{R \times 3.35}$ hemoglobin in blood (hemoglobin contains 0.335 per cent of iron), where R is the colorimeter reading of the solution tested.

Preparation of standard iron solution Dissolve 0.7 gm. pure ferrous ammonium sulphate in about 50 c.c. of distilled water. Add to the solution 20 c.c. 10 per cent iron free sulphuric acid, warm slightly and then add 0.1 N (approx.) potassium permanganate solution to oxidize the ferrous salt completely. Dilute with distilled water to 1 l. Each c.c. contains 0.1 mg. iron.

Method of determination of iron in tissues—The organ or tissue to be examined is minced and washed with water to remove blood. It is then dried in a steam oven. The dried material is then ground in a mortar until it passes through a 30 mesh sieve.

One gm. of this material is weighed into a 300 c.c. Kjeldahl flask to which are added 10 c.c. concentrated H_2SO_4 . The flask is heated in a fume chamber, gently for fifteen minutes, and then strongly for thirty minutes. At the end of this time the walls of the flask are free from charred material and its contents consist of a brown homogeneous fluid. This is allowed to cool and then diluted with an equal volume of water and transferred to a 50 c.c. stoppered graduated flask. A rise of temperature occurs on diluting and washing the fluid into the graduated flask. When room temperature is again reached the contents of the flask are made up to the mark. A certain amount of light, flocculent precipitate forms on adding water to the acid digestion fluid. The contents of the flask are therefore shaken and 5 c.c. are withdrawn immediately and placed in a Pyrex tube, to which 1 c.c. of perhydrol is added. The tube is inclined as in the previous method and the oxidation with perhydrol there outlined is followed. If much iron is present the final solution has a slight yellow color after oxidation which almost entirely disappears on cooling.

After cooling, the solution is transferred to a 50 c.c. graduated flask and acetone and thiocyanate solution added as before, a standard for comparison being also prepared as already described.

In the case of organs containing more than the normal amount of iron (where siderosis is present) 0.5 gm. should be taken instead of 1 gm., and it may be found necessary to prepare a stronger standard solution for colorimetric comparison.

Method of determination of iron in urine—To 100 cc of the twenty four hour specimen of urine placed in a 300 cc Kjeldahl flask 10 cc of concentrated sulphuric acid are added. The contents of the flask are vigorously boiled until frothing begins to take place. Heating is then carried on with caution until frothing ceases. A full flame is then used again until the walls of the flask are free from charred material and homogeneous fluid results. After cooling this fluid is diluted and transferred to a 50 cc graduated flask whose contents are made up to the mark when they have cooled again to room temperature. Ten cc of the liquid are transferred to a Pyrex tube and concentrated by boiling. Until the volume has been considerably reduced the flame of the burner must not be directed to the bottom of the tube but to the wall of the tube just below the surface of the liquid. When nearly all the water has been driven off and white fumes begin to be evolved 0.5 cc perhydrol is added as before followed by further quantities of 0.3 cc and 0.2 cc. After the last addition the resulting fluid is boiled vigorously for three minutes when practically no more fumes are evolved. The liquid is then allowed to cool, diluted with about 2 cc distilled water and transferred to a 25 cc stoppered graduated flask. One cc concentrated sulphuric acid is added and 12.5 cc acetone. The contents of the flask are made up to about 20 cc and allowed to cool to room temperature. Three cc of the thiocyanate solution are then added and the contents of the flask well mixed and made up to the mark. The standard for comparison is prepared at the same time in a similar flask using 1 cc of a solution containing 0.01 mg iron per cc, 1 cc concentrated sulphuric acid and 12.5 cc acetone. The unknown solution as thus prepared is cloudy. A portion of it is therefore immediately centrifuged in a closed tube for five minutes when the clear supernatant fluid which results is compared with the standard in the colorimeter.

The iron solution used for preparing the standard for comparison is one tenth the concentration of the standard previously used and is prepared from the latter by suitable dilution with distilled water.

It is essential in this method where very small quantities of iron are estimated to prevent dust from falling into the solutions as it has been found that the atmospheric dust at any rate in an industrial center contains sufficient iron to cause errors in the result if precautions are not taken to exclude it.

REVIEWS

Books for Review should be sent to Dr Warren T Vanghan, Medical Arts Building,
Richmond, Va

*Principles and Practice of Chemotherapy**

UNTIL comparatively recent times, the therapeutics of disease has been mainly symptomatic and, to a greater or lesser extent, empiric, modern developments in knowledge of the parasitic causes of disease and the consequent better understanding of the mechanism concerned in the production of the pathology associated with the disease resulting, have led to a definite and systematic search for specific remedies or methods of treatment to which attempts the term chemotherapy is applied

Originating largely in the work of Ehrlich which had its inception in the thought "that the ways and means by which drugs are distributed over the body must be of the greatest importance in the rational development of therapeutics," modern chemotherapy is defined by Kolmer as "The prevention or treatment of disease by chemical disinfection or inhibition of the parasitic causes without marked or serious toxic effects" upon their host

But little consideration of this definition, and but a casual survey of the literature concerned with it during the last decade alone, suffices to bring a realization of the broad field involved and the numerous, complex, and interrelated problems presented for study and consideration

Until the publication of Dr Kolmer's book, there existed nowhere in any language a systematic or comprehensive survey of this complicated subject

Had Dr Kolmer done nothing more than present a survey of the literature of modern chemotherapeutic studies he would have rendered an invaluable service to all who are concerned with the prevention and treatment of disease

He has done more than this, however Not only are the literature and studies of the world surveyed and presented in a systematic manner, but the results are reviewed and judged in the light of the comprehensive experience of a worker intimately concerned with the development of the subject

The book is divided into ten parts, all interlocking and welded into a harmonious whole

After an historic discussion, Part I considers in detail the methods for determining the toxicity of chemical agents in relation to their chemotherapeutic use Part II is a comprehensive discussion of the chemotherapy of bacterial and mycotic disease in man and the lower animals Part III discusses the chemotherapy of trypanosomal diseases Part IV discusses the chemotherapy of spirochetal diseases other than syphilis Part V takes up the chemotherapy of protozoan and metazoan diseases excluding trypanosomal and spirochetal diseases Part VI deals with the chemotherapy of diseases of unknown or doubtful etiology

These sections cover 446 pages and bring to the reader a well planned, clearly presented, and eminently practical discussion of the entire field of modern chemotherapy

*Principles and Practice of Chemotherapy with Special Reference to the Treatment of Syphilis By John A Kolmer Professor of Pathology Graduate School of Medicine University of Pennsylvania Pp 1106 82 illustrations Cloth Price \$12.00 net W B Saunders Co Philadelphia

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto

There is no specialty of medicine or surgery to which this portion of the book is not applicable, no worker who cannot find information and assistance applicable to his particular problem.

What has been done, how to do it what might be done, and practical suggestions for its accomplishment abound on every page. Nothing is left to guesswork everything is detailed. The physician, the surgeon and the laboratory worker may all profit by the material here presented.

Part VII and the remainder of the book (609 pages), are devoted to the treatment of syphilis.

There is much to support the contention that generally speaking syphilis is a very much mishandled disease. There are too many for whom the study and diagnosis of syphilis begins and ends with the Wassermann test too many whose treatment is of the disease rather than the patient with the disease too many whose scheme of treatment is that furnished by the detail man or the manufacturer of preparations for the purpose.

There are too many so-called serologists also whose knowledge is entirely technical and who fail to realize or are ignorant of the relation of the serology of syphilis to its pathology and to the variations related to its pathology and treatment.

Standard or set methods for the treatment of syphilis are impossible to formulate. The successful treatment or even an intelligent attempt involves an appreciation and understanding of all the minutiae which affect the particular case in point.

It is surely not too much to expect of any one undertaking the treatment of syphilis that he shall have some intelligent conception of the rationale underlying the various drugs available for the purpose some understanding of what they are how they act when they may and may not be used and something concerning the nature and prevention of untoward by-effects.

All this is discussed in great detail as related to all the preparations utilized in modern syphilology. The discussion of various types of reactions is a mine of information on the subject and summarizes all that can be said at present concerning their prevention or treatment and as far as the reviewer knows this information has not before been presented in so detailed and practical a manner in any one book.

While the methods used by Kolmer for the treatment of syphilis are presented in detail the necessity for the individualization of treatment is consistently emphasized and all the information available for an understanding of the subject is presented and discussed.

While of great value to the syphilographer and serologist this section of the book should prove of inestimable value to the practitioner at large for the comprehensive information it contains relative to the therapeutic value of the many methods and preparations now in use for its clear and thorough discussions of the serology of the disease and its clinical application the causes prevention and treatment of arsenical and other reactions and especially the detailed presentation of methods.

All in all the book is a worthy companion and supplement to the author's previous volume on 'Infection Immunity and Biologic Therapy' and deserves a place in the library of all who are concerned with the study treatment or prevention of disease.

The first of its kind in any language it is doubtful if this book will ever be displaced from the commanding position it now occupies with reference to its subject.

pected that in due course this same observation will be made in various directions with reference to ephedrine also. And in this connection it may be noted that the ancient Chinese used Ma Hwang as a diaphoretic (possibly without justification) and recent pharmacologic work has shown that ephedrine stimulates the stellate ganglia. There is some stimulation of the sympathetic nerve supply to the secretory glands and this also seems to depart in some degree from the typical epinephrine action. Apparently the central nervous stimulation may occasionally cause sweating in the case of toxic effects from very large doses. The unequal contractions of the various parts of the systemic vasculature would seem to produce temporarily a somewhat different distribution of blood in the various organs and parts of the body from that which follows the administration of epinephrine. Synergism has been noted between ephedrine and epinephrine and between ephedrine and tyramine.

After doses of ergotamine sufficient to produce a fall in blood pressure when epinephrine was injected Nagel produced a rise in pressure by injection of ephedrine.

The minimal lethal dose intravenously for dogs was found by Chen to be from 70 to 75 mg per kilogram of weight. From this it might be inferred that a man of 150 pounds weight would be fatally poisoned by some 60 to 70 grams if injected intravenously. In contrast to this the usual clinical dose is from 1 to $2\frac{1}{2}$ g and $6\frac{2}{3}$ g have been given in a single dose without untoward effects.

From a clinical standpoint the best results have so far been obtained when the drug was applied locally to the nose in cases of chronic congestive conditions such as hypertrophic rhinitis and hay fever. In asthma it has also been found of definite value, and in the treatment of urticaria and anaphylaxis the drug would appear to have a promising future. A rather unexpected feature of the action of ephedrine was the observation that it appeared to be the best single drug to use as a respiratory stimulant in cases of narcotic poisoning. And the drug has also a certain field of usefulness as a mydriatic.

The use of the drug as a cardiac or circulatory stimulant is still in the experimental stage and the general results in the field have not so far been as promising as might have been expected. But there is still a possibility that the substance may be found valuable in certain types of cardiac or circulatory conditions. The drug apparently cannot replace epinephrine as a local styptic for use in local anesthetic solutions for its local vasoconstricting action is too slow to prevent the general absorption of the anesthetic.

It is obvious that modern pharmacologists—and significantly enough the foremost of these is Dr K K Chen—are now interpreting the dream of Emperor Shen Nung (3217 B C) who *tasted* Ma Huang and then wrote it down in his *Pentsao*, or pharmacopeia, as a good and useful drug. Could his imperial majesty now come back to earth he would be in a position to exclaim with modern inspiration, All things come to him who waits 5000 years!

—D E J

The Menace of the Slightly Positive Wassermann

PARADOXICAL as it may seem, the obvious sometimes requires recurring or persistent reiteration before it is generally appreciated.

While the subject is not altogether new, has been discussed by various writers, and commented upon in numerous journals including this one,¹ a recent paper by Mitchell again utters a timely warning relative to the malinterpretation of the Wassermann reaction as a potent cause of syphilophobia.

"As a disseminator of false diagnosis of syphilis" says Mitchell the slightly positive Wassermann report was without a rival until the advent of the Ahlrams machine" and the paper recounts illustrations of the harm done by the erroneous interpretation of such reports.

Mitchell believes that there is a moral obligation on the part of serologists to make it clearly understood by clinicians that the Wassermann report is frequently only a symptom and that in case of doubt, further specimens should be examined.

Let it be said and emphasized that this indeed is the *serologist's* conception of the test and that it is the serologist's further contention that there is a very definite moral obligation upon the part of the clinician to become familiar with this and many other pertinent facts related to the diagnosis and treatment of syphilis—an obligation too often neglected and unrecognized.

It is unfortunate for the patient a stigma upon the profession at large a potent factor in the production of syphilophobia a still more potent factor in sowing a crop of partially treated syphilitics from whom will be reaped a generous harvest of neurosyphilitics in the years to come and a reproach to the physician in general that the introduction of the Wassermann test and the arsenicals have brought about as a by-product a number of pseudosyphilographers for whom, as Biernan² has said "The clinical study of syphilis is unnecessary. The public Wassermann laboratory makes the diagnosis and a few injections of naphthammine clear up the lesions."

The responsibility for this situation rests more heavily upon the clinician at large than upon the serologist.

Be it said to the serologist's credit that he has always emphasized the status of the Wassermann reaction as but one phase in the examination of the patient for evidences of syphilis, that he has contributed greatly to an understanding of its limitations, reiterated again and again the vital necessity for its clinical interpretation and correlation with all the other features of the particular case, and pleaded vehemently for some clinical interest and understanding of its mechanism and clinical significance.

There are, it is true, unscrupulous and mercenary serologists just as there are unscrupulous and ignorant clinicians and both should be sought out and scourged from the profession.

The subject has been discussed and rediscussed and nevertheless is still worthy of attention.

This much may be again said with confidence.

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS, MO, MARCH, 1927

No 6

Editor-in-Chief WARREN T VAUGHAN, M D

Richmond, Va

ASSOCIATE EDITORS

DENNIS E JACKSON, M D	CINCINNATI
PAUL G WOOLLEY, M D	- - LOS ANGELES
J J R MACLEOD, M B	- TORONTO
W C MACCARTY, M D	ROCHESTER, MINN
GERALD B WEBB, M D	COLORADO SPRINGS
VICTOR C MYERS, PH D	- IOWA CITY
RUSSELL L HADEN, M D	- - ROSEDALE, KAS
JOHN A. KOLMER, M D	PHILADELPHIA
ROBERT A KILDUFFE, M D	ATLANTIC CITY, N J
A PARKER HITCHENS, M D	MANILA, P L
DEAN LEWIS, M D	- - - - BALTIMORE
GEORGE R HERRMANN, M D	- NEW ORLEANS
HAROLD D CAYLOR, M D	- - ROCHESTER, MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis Mo as Second-Class Matter

EDITORIALS

Ephedrine

IT HAS been a long time since the question was first asked, "Can any good come out of Nazareth?" In a somewhat similar spirit the question might have been asked only a few years ago, Can any good come out of Chinese medicine? Whether by chance or by actual scientific trial we cannot say, nevertheless we can now answer the latter question with a positive yes, for from out of the superstition, the medical huff-duff and the folklore of more than five thousand years a new drug—for us—ephedrine has emerged. As early as 1885 and 1887 this drug (or an alkaloid supposed to be ephedrine) had been isolated by Yamanashi and Nagai from *ephedra vulgaris*, var *helvetica*. The popular name of this rather common Chinese plant is Ma Huang and other alkaloids are also present in it. The earlier pharmacologic work on ephedrine was somewhat confusing and the investigations were, unfortunately, not followed up as they should have been.

Structurally the drug is rather similar to epinephrine and tyramine and like them it possesses sympathomimetic properties. Beginning with Amatsu and Knabota in 1917 and Chen and Schmidt about 1923 a rapidly growing list of pharmacologists and chemists have carried out a brilliant and very promising series of experiments on this drug. Already an important future for this alkaloid is definitely indicated. It is practically certain to displace the use of epinephrine in certain types of cases. And in all probability it will find a use in new fields of its own. The pharmaceutical drug manufacturers have already begun to read the signs of the future with reference to this compound and it appears that various manufacturers in this country have now had in stock, or are trying to get, a goodly supply of the crude Ma Huang. These companies have commendably proceeded with caution with reference to the new drug. For notwithstanding the many interesting and valuable points which have already been discovered about this substance, it appears that more is yet to follow.

Briefly stated the drug has been shown to raise the blood pressure, cause dilatation of the pupil, contract the uterus, relax the intestinal and bronchial musculature, increase the blood sugar, stimulate the heart under some conditions and apparently depress it under others, to stimulate the central nervous system in some degree, to cause death by heart failure and to possess a relatively low toxicity. The rise in blood pressure produced by ephedrine is neither so abrupt nor so high but lasts very much longer than that produced by epinephrine. And ephedrine solutions are very stable, can be sterilized by boiling and the drug can be effectively administered by stomach as well as hypodermically. It appears that neither animals nor man develop any special tolerance for the drug when it is administered in repeated doses from day to day over comparatively long periods. But on intravenous injection it is found that the first dose if it be large enough will produce a maximum rise of pressure, the second dose (of the same amount) will then raise the pressure only one half or two thirds as high as the first and following doses, if given within ten or fifteen minutes of each other, tend progressively to produce less and less of a rise in arterial pressure and usually the later doses may produce some temporary fall in pressure. In a number of recent experiments the writer has found that when this stage has been reached, and ephedrine in any sized dose will no longer produce a rise in pressure, then an injection of epinephrine will promptly raise the pressure almost as high as the given dose would have raised it before any ephedrine had been given. This observation can only mean that ephedrine and epinephrine do not act on identically the same structures, or else that the two drugs do not affect them in the same identical way. Many years ago Barter and Dale investigated the pharmacologic action of a large number of compounds to which they gave the name of "sympathomimetic amines." These authors showed that the relative action of the various members of the series on diverse organs and structures innervated by the true sympathetic nerves varies considerably and that a member which was very active on one organ might not manifest this same degree of activity on another, presumably similarly innervated structure or organ. It is to be ex-

pected that in due course this same observation will be made in various directions with reference to ephedrine also. And in this connection it may be noted that the ancient Chinese used Ma Huang as a diaphoretic (possibly without justification) and recent pharmacologic work has shown that ephedrine stimulates the stellate ganglia. There is some stimulation of the sympathetic nerve supply to the secretory glands and this also seems to depart in some degree from the typical epinephrine action. Apparently the central nervous stimulation may occasionally cause sweating in the case of toxic effects from very large doses. The unequal contractions of the various parts of the systemic vasculature would seem to produce temporarily a somewhat different distribution of blood in the various organs and parts of the body from that which follows the administration of epinephrine. Synergism has been noted between ephedrine and epinephrine and between ephedrine and tyramine.

After doses of ergotamine sufficient to produce a fall in blood pressure when epinephrine was injected Nagel produced a rise in pressure by injection of ephedrine.

The minimal lethal dose intravenously for dogs was found by Chen to be from 70 to 75 mg per kilogram of weight. From this it might be inferred that a man of 150 pounds weight would be fatally poisoned by some 60 to 70 grains if injected intravenously. In contrast to this the usual clinical dose is from 1 to $2\frac{1}{2}$ gr and $6\frac{3}{4}$ gr have been given in a single dose without untoward effects.

From a clinical standpoint the best results have so far been obtained when the drug was applied locally to the nose in cases of chronic congestive conditions such as hypertrophic rhinitis and hay fever. In asthma it has also been found of definite value, and in the treatment of urticaria and anaphylaxis the drug would appear to have a promising future. A rather unexpected feature of the action of ephedrine was the observation that it appeared to be the best single drug to use as a respiratory stimulant in cases of narcotic poisoning. And the drug has also a certain field of usefulness as a mydriatic.

The use of the drug as a cardiac or circulatory stimulant is still in the experimental stage and the general results in the field have not so far been as promising as might have been expected. But there is still a possibility that the substance may be found valuable in certain types of cardiac or circulatory conditions. The drug apparently cannot replace epinephrine as a local styptic for use in local anesthetic solutions for its local vasoconstricting action is too slow to prevent the general absorption of the anesthetic.

It is obvious that modern pharmacologists—and significantly enough the foremost of these is Dr K K Chen—are now interpreting the dream of Emperor Shen Nung (3217 B C) who *tasted* Ma Huang and then wrote it down in his *Pentsao*, or pharmacopeia, as a good and useful drug. Could his imperial majesty now come back to earth he would be in a position to exclaim with modern inspiration, All things come to him who waits 5000 years!

—D E J

The Menace of the Slightly Positive Wassermann

PARADOXICAL as it may seem the obvious sometimes requires recurring or persistent reiteration before it is generally appreciated

While the subject is not altogether new, has been discussed by various writers, and commented upon in numerous journals including this one,¹ a recent paper by Mitchell again utters a timely warning relative to the misinterpretation of the Wassermann reaction as a potent cause of syphilophobia

As a disseminator of false diagnosis of syphilis says Mitchell the slightly positive Wassermann report was without a rival until the advent of the Abrams machine and the paper recounts illustrations of the harm done by the erroneous interpretation of such reports

Mitchell believes that there is a moral obligation on the part of serologists to make it clearly understood by clinicians that the Wassermann report is frequently only a symptom and that in case of doubt further specimens should be examined

Let it be said and emphasized that this indeed is the *serologist's* conception of the test and that it is the *serologist's* further contention that there is a very definite moral obligation upon the part of the clinician to become familiar with this and many other pertinent facts related to the diagnosis and treatment of syphilis—an obligation too often neglected and unrecognized

It is unfortunate for the patient a stigma upon the profession at large a potent factor in the production of syphilophobia a still more potent factor in sowing a crop of partially treated syphilitics from whom will be reaped a generous harvest of neurosyphilitics in the years to come and a reproach to the physician in general that the introduction of the Wassermann test and the arsenicals have brought about as a by product a number of pseudosyphilographers for whom as Bieman² has said The clinical study of syphilis is unnecessary The public Wassermann laboratory makes the diagnosis

and a few injections of arsenphenamine clear up the lesions

The responsibility for this situation rests more heavily upon the clinician at large than upon the serologist

Be it said to the serologist's credit that he has always emphasized the status of the Wassermann reaction as but one phase in the examination of the patient for evidences of syphilis that he has contributed greatly to an understanding of its limitations reiterated again and again the vital necessity for its clinical interpretation and correlation with all the other features of the particular case and pleaded vehemently for some clinical interest and understanding of its mechanism and clinical significance

There are it is true unscrupulous and mercenary serologists just as there are unscrupulous and ignorant clinicians and both should be sought out and scourged from the profession

The subject has been discussed and rediscussed and nevertheless is still worthy of attention

This much may be again said with confidence

As long as there are physicians for whom a Wassermann test is a Wassermann test regardless of how or by whom performed, as long as the physician selects his serologist without careful consideration of his ability, technical skill, and professional standing, as long as physicians vary in the thoroughness of their knowledge and understanding of syphilis in general and especially its serology, as long as any one can open a laboratory, proclaim himself a serologist, and have his reports accepted without question, as long as physicians neglect to choose a serologist who can be, when required, a consultant, and as long as laboratory reports are made to take the place of careful, painstaking, and intelligent studies of each individual case—just so long will errors, fallacies, and clinical misinterpretations leading to syphilophobia and worse evils be perpetuated.

As has been said before in these pages² "The acceptance of a Wassermann report at its 'face value' indicates a lack of understanding of the factors influencing the occurrence and detection of the reaction, and an equally serious lack of appreciation of the essential necessity for a careful study of each case upon its individual merits."

REFERENCES

- ¹Kilduffe, R. A. The "Face Value" of the Wassermann Report, *JOUR LAB AND CLIN MED*, November, 1925, 21, 2
- ²Mitchell, J. H. The Menace of the Slightly Positive Wassermann Reaction, *Jour Am Med Assn*, October 23, 1926, LXXXV, 1351
- ³Biegan, C. J. A Thorough History An Important Factor in Syphilis, *Am Jour Syph*, 1921, v, 565

—R. A. K.

United States Public Health Service

The Weekly Public Health Reports of the United States Public Health Service are now available to all persons in the United States and its possessions, Canada, Cuba, and Mexico, for the nominal subscription of \$1.50 per year, it was announced in the current issue of the official bulletin of the Post Office Department.

The announcement states that these reports will be sent with postage prepaid to each subscriber, and represents an innovation by the Public Health Service to further increase knowledge about public health and sanitation. These Reports contain information as to the world prevalence of disease, and each issue has special articles by experts on sanitation.

Subscriptions should be accompanied by money orders, and sent to the Superintendent of Documents, Government Printing Office, Washington, D. C.

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN M.D. Editor in Chief
Medical Arts Building
Richmond Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. LACLEOD M.B.
University of Toronto Toronto

Clinical Pathology

W. C. MACCARTY M.D.
Mayo Clinic Rochester Minn.

Biochemistry

VICTOR C. MYERS Ph.D.
State University of Iowa Iowa City

Immunology

JOHN A. KOLMER M.D.
University of Pennsylvania
Philadelphia

Bacteriology

A. PARKER HITCHENS M.D.
Bureau of Science Manila, P. I.

Internal Medicine

GEORGE R. HERRMANN M.D.
Tulane University New Orleans

Pathology

PAUL G. WOOLLEY M.D.
Los Angeles

Tuberculosis

GERALD B. WEBB M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR M.D.
Mayo Clinic, Rochester Minn.

Experimental Medicine

RUSSELL L. HADEN M.D.
University of Kansas Rosedale

ROBERT A. KILDUFFE M.D.
Atlantic City N. J.

Surgery

DEAN LEWIS M.D.
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523 25 PINE BLVD., ST. LOUIS.

Entered at the Post Office at St. Louis Mo. as Second Class Matter

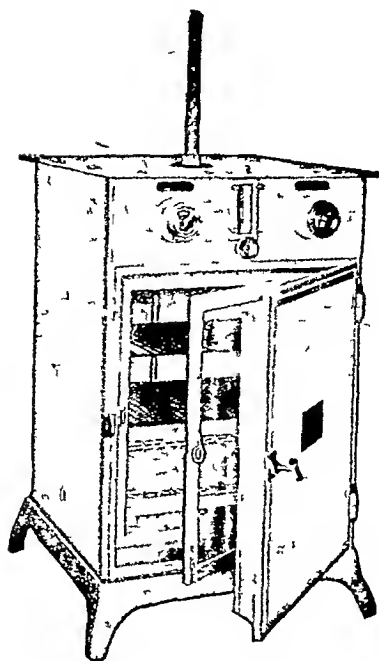
LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment. Estimates Cheerfully Furnished. Prompt Service.

See our advertisements pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA, PA.



Freas Incubator No 26--

Your Incubator Must be Accurate

Just as with your laboratory worker your incubator must be capable of performing the assigned tasks accurately

Accuracy in an incubator is its ability to maintain the temperature constant continuously

This is why the Freas Incubators are used in the leading laboratories

The Thermo Electric Instrument Co.
1215 South Grove Street, Irvington, N. J.

FREAS

ELECTRIC INCUBATORS

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO., APRIL 1927

No 7

CLINICAL AND EXPERIMENTAL

ON NEPHLOMETRY*

By HANS KLEINMANN, M.D. PH.D., BERLIN, GERMANY

I. PREFACE

ADVANCES in physiologic chemistry are connected with methods allowing of estimating accurately and at the same time rapidly the strength of very dilute solutions of inorganic as well as organic substances. For only by such methods is it possible to make series of experiments with biologic liquids, i.e., blood or serum such as are necessary for the investigation of physiologic and pathologic processes.

During the last few years investigators have therefore striven to develop special analytic methods for biologic work. Optical methods have proved especially suitable and a large number of analytic processes based on colorimetric estimation have been described.

Besides these methods based on the estimation of the intensity of coloring, called forth in solutions by chemical reactions, an altogether independent branch of optical analysis has been developed which is based on the comparing and gauging of the turbidity of solutions. It has therefore been given the name of nephelometry.

In colorimetric analysis the intensity of the coloring of a solution produced by a certain reaction is used to gauge the strength of the solution by comparing it with a solution containing the reacting substances in known quantity (a standard solution). In similar manner the strength of a solution is estimated nephelometrically, not it is true, by means of a reaction producing color but of a reaction producing turbidity by comparing the turbidity produced in a solution of the same substance of known strength.

From the Chemical Department of the Pathological Institute of the Charité Berlin
Received for publication October 3 1926

In recent years a series of papers on nephelometry has been published. Reactions producing turbidity have been described and apparatus designed by means of which turbid solutions could be compared and estimated, and much careful work has been devoted to ascertaining what relation exists between the turbidity produced and the quantity of the substance producing it and how that quantity may be calculated from the data obtained in gauging the turbidity.

The author has, by a series of investigations begun in 1919¹ and continued since then, shown that the relation between turbidity and concentration is very simple and that under certain working conditions turbidity and concentration are directly proportional. He succeeded in proving that nephelometry may be employed in the same manner and to the same purpose as the allied science of colorimetry.

This analogy between colorimetry and nephelometry is moreover completely in accordance with theoretical considerations on the nature of Tyndall light, for it is this kind of light that is measured in nephelometric analysis.

The laws of nephelometry could not, however, be established on a firm basis, nor could nephelometric analysis be carried on successfully, unless an instrument, a nephelometer, could be designed, in which the optical defects of the hitherto known apparatus, the real cause of the discrepancies between the results of analytical work and the simple laws governing the phenomena, were avoided.

The author has designed an instrument of this kind and has, by means of it, succeeded in fully confirming by practical analysis the laws of nephelometry deduced from theoretical considerations.

With the help of this new type of nephelometer, a large number of nephelometric methods were either tested or newly developed. The author succeeded in finding and developing nephelometric methods suitable for biochemical analysis as well as for investigations on ferments, and applicable also to purely chemical work on colloidal substances.

The following pages contain a summary of the results of the work done in the last few years. The theory of nephelometry and its most important applications will be further discussed and the apparatus and methods described.

II THEORY OF NEPHELOMETRY

Richards and Wells² were the first to make a long series of quantitative nephelometric investigations. These authors, who worked with a primitive instrument of their own construction, state the maximum error in nephelometric analysis to be 5 per cent and that within this limit they had found turbidity and concentration to be proportional. Their work, however, shows how difficult accurate nephelometric measurements were at that time, how uncertain the results were, and how high the limit of possible error had to be drawn. Nephelometric analysis was subsequently developed further, mainly by Americans. Kober⁴ as well as Bloor⁵ designed instruments which by a simple manipulation could be changed from a Duboseq colorimeter into a nephelometer. All these investigations, however, seemed to show that turbidity and concentrations are not proportional. Even with solutions of a strength

nearly approaching that of their standard solution they state that they had found results incompatible with the principle of proportionality and their results finally led them to establish a complicated mathematical formula from which the concentration of the solution could be calculated after measurement, or determined graphically, by means of a diagram. Their results and conclusions were, however, at variance with the theoretical considerations of Rayleigh on the intensity of the diffracted light emitted by a Tyndall strip. According to Rayleigh the intensity of the diffracted light is

$$J = \frac{c \cdot v \cdot k}{y \cdot s}$$

J being the intensity of the light, c the concentration, v the volume of the particles, s their specific weight, y the wave length of the light and k a constant.

Now in comparing two turbid solutions, the values v , k , v and s cancel each other and we therefore have

$$J/J = c/c_1$$

or in words *Provided the suspended particles are of equal size, the turbidity of the two solutions is proportional to their concentration.*

Nevertheless it was of course quite possible that some source of error caused the actual measurements to diverge more or less from this law.

Investigations the particulars of which cannot be entered into here¹ and to which we must therefore refer the reader showed however that the Duboseq colorimeter when used as a nephelometer as was done by Kohler and Blooi, possesses several optical defects to which the discrepancies between their results and the relation between turbidity and concentration demanded by theory, are due.

The writer, therefore aimed at designing an instrument in which the defects of the Duboseq colorimeter when used as a nephelometer would be avoided. With the assistance of the firm of Schmidt and Haensch, Berlin he succeeded in constructing a new type of nephelometer fulfilling this condition.

The results obtained in working with this instrument soon showed that certain conditions being duly observed turbidity and concentration are accurately proportional. This law was confirmed again and again in a series of investigations carried out with a great variety of reactions producing turbidity.

The high accuracy of the measurements obtained with this new apparatus as well as its handiness and the simplicity of its design enables us to place nephelometry on a level with colorimetry as an analytical method.

The conditions which must be observed in nephelometric investigations will be specified in detail further on. Results showing the accuracy of measurement obtainable with the instrument will be given and its application to various kinds of analytical work discussed.

We will first give a description of the nephelometer its modifications and its manipulation.

III DESCRIPTION OF THE NEW TYPE OF NEPHELOMETER²

(a) *The Macronephelometer*—The working principle of this instrument consists in producing Tyndall-cones (the height of which can be varied) in two turbid media arranged side by side and measuring and comparing their luminous intensity in a line perpendicular to their axis by means of suitable optical fittings. The only difference between this instrument and the instruments usually employed for colorimetric measurement, therefore, is that the light, the intensity of which is measured, is diffracted, not transmitted, light. Thus it is possible to apply the principle, familiar to us from colorimetric methods, of varying the luminous intensity in fixed proportions by varying the "height or thickness of the layer," in our case the diameter of the Tyndall-cones. The concentration of two turbid solutions should therefore,

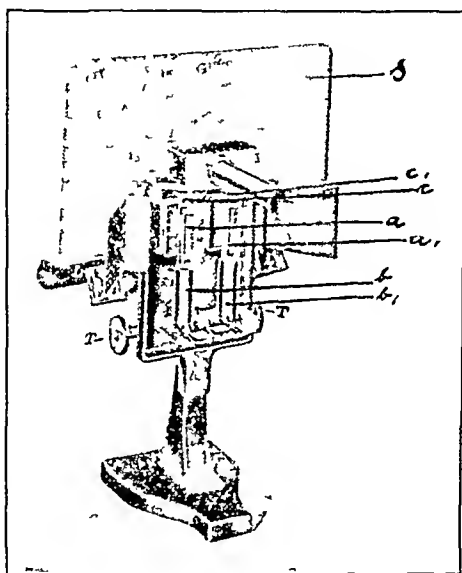


Fig 1

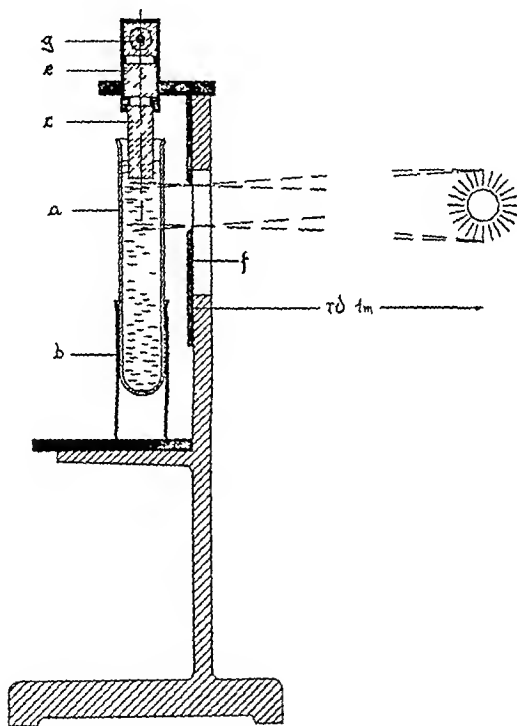
if the law of proportionality holds good, as in colorimetric measurements, be inversely proportional to the diameter of the Tyndall-cones produced in them, when reduced to equal luminous intensity. We must, however, keep in mind that in turbid media the intensity of the Tyndall-light increases with the concentration, owing to the increase in the number of the diffracting particles, whereas in colorimetric analysis the luminous intensity on the contrary decreases with concentration. From the nephelometers hitherto constructed on the same principle, that of the applied Duboseq colorimeter, the present instrument differs mainly in certain modifications by which the optical and technical defects peculiar to these instruments, the nature of which I have explained elsewhere, are avoided.

The new nephelometer is shown in the annexed illustrations of which

*This instrument as well as all accessories will be supplied by the firm of Schmidt & Haensch, Berlin or by American Kreuger & Toll Corporation, New York City, 114 115 Liberty Street.

Fig. 1 is an objective front view, Fig. 2 a diagrammatic sketch of the design in side elevation

Fig. 1 and Fig. 2 show the two test tubes a and a_1 into which the standard solution and the solution to be tested are filled. They hold about 12 cc each. These test tubes are carried in metal casings b and b_1 , in which they



Fig

fit easily so that they can be moved up and down without difficulty. The casings are fitted on spring braces adapted to slide in a suitable frame.

A beam of light is thrown on the test tubes by a lamp placed in front of the instrument and the Tyndall cones thus produced are observed and gauged in a line perpendicular to the axis of the beam.

For this purpose the diffused light is made to pass first through two solid glass cylinders c and c_1 identical in shape and size and cut out of adjacent parts of the same block of glass in order to render their action on the light absolutely symmetrical. To eliminate the error which may be caused

by observing the surface of the liquid, the lower parts of the cylinders are immersed in it. By a suitable arrangement of diaphragms the cylinders receive light only from the central part of the Tyndall-cones.

The section of the tubes exposed to light and therefore also the diameter of the Tyndall-cones can be varied at will by varying the height of the windows f and f_1 through which the light reaches the turbid solutions. These windows are about 4.5 cm high and about 2 cm wide. The bottom part of the window openings is closed by a movable metal plate with a sharp edge, fitted on the interior surface of the wall of the instrument, so that the shadow limiting the illuminated section is very sharply defined. These metal shutters can be displaced by means of rack and pinion and the displacement read by means of verniers. The height of the windows can be varied independently for each Tyndall-cone, by means of the corresponding milled screw heads t and t_1 , from complete closure to the full height of 4.5 cm.

The verniers may be conveniently read from the back, the observer's side, of the instrument in the prisms L and L_1 , that receive light from the observation lamp and are adapted to be moved laterally to bring the scale into focus.

The scale is divided into millimeters and reads from zero (closed window) to 45 (window opened full). By means of the verniers the scale allows reading of 0.1 mm. The observer is screened from the light of the lamp by a removable screen s . In order to exclude the light reflected from surrounding objects the turbid solutions are enclosed in a box of blackened sheet metal, not shown in the drawing, which is permanently fitted on the instrument and can be easily opened and closed.

A frosted Osiam lamp of 100 candle power is preferably employed as source of light. It should be installed at a distance of 75 cm in front of the instrument in a line with its optical axis and on a level with the windows.

(b) *The Micronephelometer*.—In working with material of which only small quantities are available, our instrument showed a disadvantage not present in the previously known form of nephelometer in which about 12 cc were required to fill the vessels used for measuring.

I, therefore, set about to design a modified form of his instrument which would allow the examination of smaller quantities of solution. In cooperation with the firm of Schmidt & Haensch I constructed a supplementary fitting to be used in the above-described nephelometer in place of the test tubes containing the solutions. By means of this modification the instrument may be used both as macronephelometer (taking 12 cc of solution) and as micronephelometer, taking smaller quantities, down to 2.6 and 1.5 cc.

For this purpose the test tubes R_1 and R_2 , Fig. 3, of smaller diameter and shorter than the tubes ordinarily used, are provided, R_1 holding 2.6 cc of solution, R_2 1.5 cc. The latter is fitted with a glass stopper. Two glass cylinders for submersion in the solution are further provided, of smaller diameter than the cylinders ordinarily used, to fit the narrower test tubes and adapted to be fitted in their places by screw heads. A diaphragm M interposed in the path of the rays adapts the latter to the reduced diameter of the

*Supplied by the firm of Schmidt & Haensch, Berlin, Prinzessinnenstr. 16 or by American Krueger & Toll Corporation, 114-118 Liberty Street, New York City.

Suitable standard solutions for comparison are, however, in some cases difficult to procure (e.g., certain solutions of proteins). In such cases and also in chemical investigations of colloidal substances, e.g., of the variation of turbidity of the solution of a colloid during a certain period of time, it is indispensable to have a fixed and invariable standard of turbidity.

Such a standard should have the following characteristics:

- 1 Its strength must, when once gauged, remain constant for an unlimited period of time
- 2 Its strength, or the amount of light it emits, must be capable of variation to any desired extent

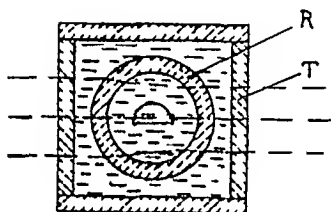


Fig. 4

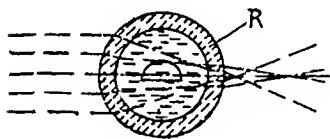


Fig. 5

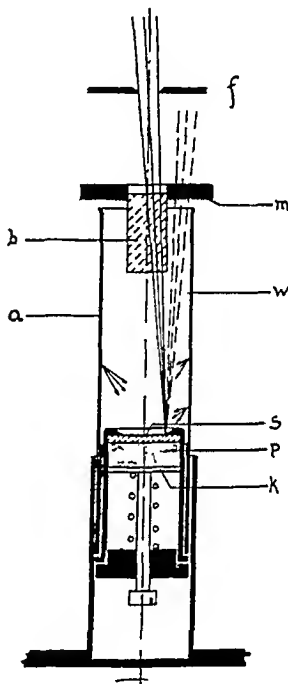


Fig. 6

- 3 Its color must also be capable of variation, so that it may be made to match the shade or tint of the various turbid solutions exactly

This latter adjustment is of importance, as the various turbid solutions, even those called white, although they are almost always of a bluish white color, vary considerably in shade from blue to yellow. In order to be able to adjust turbid solutions accurately to equal brightness, it is absolutely necessary to have them of the same color or even shade of color, unless monochromatic light is available. There is, however, some difficulty in producing monochromatic light of sufficient brilliancy, not to speak of the inconvenience of manipulating the necessary apparatus.

A standard of turbidity consisting of a liquid or of a solution cannot fulfill the above conditions. The author therefore turned his attention to other methods of preparing a suitable and sufficiently variable standard of turbidity and finally attained his object in the following manner:

The walls of a test tube, such as is used to hold the solution in the nephelometer, are frosted in a way that allows of varying the thickness of the frosting layer or coating.

When a tube frosted in this manner is placed in the nephelometer, the frosted white surface throws diffuse light on the bottom of the tube. On the bottom of the tube a colored preparation is spread the color of which can be varied as required. This preparation reflects colored light upwards into the immersed cylinder of the nephelometer.

The standard of turbidity prepared on this principle took the practical form shown in Fig 6, *a* is a small glass tube which in order to avoid light, reflected from its walls penetrating upwards in to the submerged cylinder, is rather wider than the ordinary nephelometer tubes. The tube (*a*) is fitted on a short brass tube indicated by strong lines in the drawing. Into this brass extension of the glass tube a cartridge is introduced from below, which will be described in detail further on and which contains the colored preparation. The whole is fitted into the base of the nephelometer tube in the same way as an ordinary tube and raised until the top of the tube encloses the glass cylinder (*b*).

The frosting of the surface of the tube is done by pouring over it a solution of collodion in ether in which an indifferent finely divided powder, such as talcum, is suspended. This solution on drying leaves a film of uniform thickness covering the surface of the glass. In this manner any desired degree of frosting can be produced by increasing or diminishing the quantity of talcum or other matter, suspended in the solution. Before the above operation is begun the brass tube end is closed by an accurately fitting brass stopper to prevent the solution from penetrating into the interior of the tube.

When the collodion film is dry, a cartridge adapted to form the colored reflecting bottom of the standard tube is introduced into the brass tube end in place of the stopper. This reflecting bottom is formed by a powder the color of which can be varied as required and which fills the top part of the cartridge. This latter is closed off by a cover glass (*s*) ground opaque, against which the powder is pressed by a piston (*h*). The piston (*h*) is kept in place by a spring and forms a uniform and evenly colored surface as bottom for the standard tube.

The colored powder used to form this bottom should be as fine grained as possible. It will in most cases be of a bluish green tint as most of the turbid solutions are bluish white. I have found a mixture of talcum sulphate of copper and blue litmus very suitable but, of course any desired colored powder may be employed for the purpose. The color that is most suitable for the turbid medium in question must be found by experiment. The right shade can always be ascertained by a few experiments.

In case the light emitted by the colored bottom is not sufficiently intense to impart the right tone of color to the white light emitted by the wall of the glass tube the collodion film itself may be colored or the tube may be lined with colored tissue paper. In this latter manner any desired color may be produced for the investigation of colored colloidal solutions.

In place of a glass tube which the investigator may himself frost as desired, Messrs Schmidt & Haensch also supply a brass tube whitened inside and provided with a longitudinal slit on the side turned towards the window of the nephelometer to allow the light to fall on the whitened interior surface of the tube.

The intensity of the light emitted by the standard of turbidity may be modified, not only by varying the density of the frosting film and the brilliancy of the reflecting bottom, but also, and even more easily, in the same way as that of ordinary turbid solutions, by adjusting the nephelometer window in front of the standard tube.

The standard of turbidity should not, however, stand freely in the nephelometer as shown in the drawing, but should be raised until it touches the top fitting (*m*), in order that the bottom may always be at the same distance from the cylinder (*b*).

The immersed cylinder receives light exclusively from the bottom of the tube, that is from the cover-glass (*s*). The dimensions of the tube are such that no light entering laterally through the walls of the tube can reach the cylinder directly.

A standard of turbidity prepared in the above manner can be varied in luminous intensity and color so as to match any turbid solution and will keep indefinitely without changing.

It will probably prove useful not only in ordinary nephelometric work but also in the investigation of kinetic processes in colloidal bodies.

IV USE OF THE NEPHELOMETER DESCRIBED ABOVE

Measurements with the new nephelometer are carried out in the following manner.

It has been proved by repeated and careful measurements that the instrument, when installed symmetrically to the source of light, is in absolute optical equilibrium, that is, both test tubes being filled with the same turbid solution and the windows adjusted to the same width of opening, both tubes show the same luminous intensity, and this state of things is not altered by exchanging the tubes. The luminous intensity of the tubes may therefore be used to adjust the source of light symmetrically in front of the instrument.

The source of light is installed as accurately as possible on a level with and symmetrically to the windows, both test tubes are filled with the same solution, both windows adjusted to the same width of opening and then instrument and lamp are carefully adjusted, so as to give uniform brilliancy over the entire field of view. The tubes a_1 and a_2 are then exchanged and, if the field of view remains unchanged, the position of the lamp and the instrument is marked on the table with chalk or a colored pencil.

Should the field of view not remain uniform in brilliancy on exchanging the test tubes, the adjustment of lamp or instrument must be repeated until the tubes can be exchanged without producing any difference.

The instrument is now ready for use.

The tubes are pressed down into the metal casing and removed out of the slides with the same. Then they are carefully cleaned outside with a chamomile

cloth The inside of the tubes should be cleaned and dried only when a series of experiments is concluded and the instrument is to be put aside for the time being Brushes or cloths invariably leave small fibers, etc., on the surface of the glass and it is, therefore, not advisable to do anything more to the instrument while in use than to rinse the tubes well with the solution to be tested When filled they are again fitted into the slides and raised until the solid cylinders are immersed in the solution Great care should also be taken to keep the cylinders clean, as impurities are very apt to cause changes in colloidal solutions Flaky precipitations were indeed often observed to form in the neighborhood of the cylinders as a consequence of insufficient cleaning Care must also be taken that no air bubbles lodge under the ends of the cylinders, either in dipping them into the solution or later on in case the temperature of the solution should rise in the course of the experiment

The position of one of the sliding shutters being fixed and noted the other is adjusted by means of rack and pinion until the luminous intensity is equal over the whole field of view The concentration of the solutions is then in inverse proportion to the openings of the windows as read on the verniers

The instrument indicates variations in luminous intensity with a high degree of precision A displacement of the shutter of 0.1 mm is distinctly observable, even with less turbid solutions

Here however not only the actual performance of the instrument but also the subjective efficiency of the observer plays an important part

The human eye which at first is insensible to fairly large differences in brilliancy by continuous practice learns to distinguish the most delicate differences

Thus the accuracy of the results attained in the first experiments may be greatly increased by practice

The subjective accuracy of observation must however, be taken into account not only generally but for every single measurement

It is absolutely necessary to give the eye time five to six minutes to adapt itself to the darkness before beginning to observe But even afterwards, in the course of a longer series of measurements it is advisable to let the eye rest from time to time in total darkness as variations in its sensitivity may vitiate the results To avoid errors from this cause it is advisable to carry out each adjustment several times say ten times which can be done rapidly and easily and to take the mean value of the observations as final result In this way the observer can eliminate the subjective errors of observation with almost absolute certainty On the other hand we must repeat here that it is absolutely necessary to test the adjustment of the nephelometer and the source of light before commencing operations by filling both tubes with the same solution as described above, even if their positions have been marked on the table previously

The accuracy of measurement which may be attained with this instrument is very satisfactory The average error is about 1 per cent and by practice this average may even be brought down to 0.5 per cent

V GENERAL DIRECTIONS FOR NEPHELOMETRIC INVESTIGATIONS

There are certain precautions that must be observed in nephelometric analysis, if successful and accurate measurements are to be obtained. These conditions which are partly analogous to those necessary in colorimetric analysis are the following:

1 The degree of turbidity of the solution to be compared must remain constant within the period of observation, nor should the solution be subject to any changes of state such as the formation of flaky precipitations. This condition is a matter of course and needs no further explanation or comment.

2 Turbid media that are to be gauged nephelometrically must be absolutely homogeneous at least to the naked eye. There is also an upper and a lower limit to the density of solutions susceptible of accurate measurement which may be easily determined empirically. If the turbidity of a solution is so slight that even a powerful beam of light produces only a faintly luminous Tyndall cone in it, the measurements are naturally less accurate than when the Tyndall-light is of ordinary intensity. On the other hand solutions of too great density are very liable to form flaky precipitations, not to speak of the absorption of the diffracted light by the superposed liquid which in turbid solutions of high density begins to be perceptible. This shows us the necessity of studying systematically every reaction producing turbidity which we wish to use in nephelometric investigations, as to the limits of concentration which may be employed, the stability of the turbid solution, etc.

3 The difference in the turbidity or, what comes to the same thing, the concentration of solutions which are to be gauged nephelometrically should not exceed the ratio of 1:4. This is merely an empirical rule embodying the experience that with solutions more widely different in concentration measurement becomes more difficult and less accurate.

In this connection it may be noted as a most interesting fact that Lednicky⁷ who, on the basis of diagrammatic sketches of the design of the above nephelometer, discussed the theory of nephelometrics mathematically and was led by his calculations to the same conclusions regarding the concentrations of the solutions to be gauged.

The difference in concentration of 1:4 is, however, so great that it will never be exceeded or reached in practice. As a solution of unknown concentration is generally compared with a standard of turbidity, the difference of 1:2 between the solutions of a turbid media will hardly be exceeded in practice. Besides a greater difference can be easily corrected by diluting the stronger solution. The slighter the difference in density is between the turbid media that are to be compared, the easier will the measurement be and the more accurate the result.

4 The turbid solutions subjected to analysis must possess equal dispersive power. For, according to the formulas established by Rayleigh, the intensity of the Tyndall-light depends on two factors:

- (a) The number of particles present in the turbid solution, and
- (b) Their size, that is, the dispersive power of the solution.

In order to enable us to determine one of these variables the other must be constant. As we wish to measure the concentration of a solution by comparison with another, that is the number of particles their dispersive power must be equal.

This condition seems very difficult to fulfill it is, however, quite possible to realize it for a number of reactions producing turbidity. In the case of inorganic substances producing turbidity, such as chloride of silver and the like, solutions of equal dispersive power can only be prepared by special devices, such as the addition of a protective colloid. With substances possessing a very large molecule such as albuminoids, alkaloids, fatty substances high up in the series, it is however comparatively easy to produce turbid solutions of equal dispersive power. That this condition is actually fulfilled is proved by the fact that a substance entering into reaction in the same concentration repeatedly gives solutions of equal turbidity.

This also shows the necessity of studying a reaction methodically in all respects, before nephelometric methods can be used successfully in connection with it.

5 The optical fittings of the instrument employed in measuring must be faultless in design and construction.

VI RESULTS OBTAINED WITH THE NEW TYPE OF NEPHELOMETER NEPHELOMETRIC METHODS

In order to ascertain the relation obtaining between the concentration of a solution and the turbidity produced solutions rendered turbid by glycogen, chloride of silver, lecithin and other like substances prepared by diluting an original solution in various proportions were measured by means of the above described instrument.

The positive and invariable result of all these measurements was that up to the limit ratio of concentration 1:4, measurable with this instrument concentration and turbidity are absolutely proportional.

As a typical instance among the long series of experiments made the following may be quoted here:

Glycogen was dissolved in distilled water in such quantity as to render the solution distinctly turbid. The solution was filtered and portions diluted in the ratios marked by the whole numbers within the limit ratio 1:4. The diluted solutions were then compared with each other in irregular sequence.

The maximum error of measurement to which a beginner is liable in his first experiments is 1 per cent. With a little practice the mean error need not exceed 0.5 per cent.

The instrument itself does not show any variations in its optical equilibrium such as are experienced in the applied Duboscq colorimeter.

The manipulation of the instrument which is small and easily movable is as simple as possible.

As the reactions producing turbidity are exceedingly sensitive they allow of determining extremely small quantities of the substances in ques-

TABLE I
SOLUTION OF GLYCOGEN DILUED IN THE RATIOS
a b c d e=2 4 6 8 10

	SOLUTION B	SOLUTION C	SOLUTION D	SOLUTION E	MEAN ERROR
Average	20 06	10 02	10 12	15 0	0 3
Compared with	a=40	a=30	a=40	c=25	0 2
Ratio of dilution	1 2	1 3	1 4	3 5	+0 2
Error	0 3%	0 2%	1 2%	0 0%	0 0%
					0 42%

tion (0 0005 mg P_2O_5 , 0 05 mg Ca, etc) We are thus enabled to establish an entire analytical system for microchemical, especially biologic purposes

The analytic results obtained are, provided the above-mentioned conditions are adhered to, wholly reliable and accurate and the mean error is almost always under 1 per cent And finally the nephelometric methods are so simple and so rapidly carried out that hundreds of estimations can be made in a few hours and thus series of experiments become possible that would be totally impracticable, if they were to be carried out by the ordinary analytic methods As an example of nephelometric analysis, the estimation of phosphoric acid¹ may be quoted, which has been generally adopted in biologic work and has been used, e g, by Willstaetter in his work on ferments for the estimation of extremely small quantities of phosphoric acid The analytic method allowing the estimation of extremely small quantities of calcium, described by P Rona and H Klemmann¹² has also proved useful in a biologic work The same authors have further elaborated a method for the analysis of proteins in extremely small quantities¹³ which has proved useful in following the process of fermentation By means of this method the action of pepsins on serum-albumin could be successfully studied, an investigation, which had hitherto offered great difficulties, owing to the want of a suitable analytic method¹⁴

P Rona and H Klemmann¹⁵ have developed a nephelometric method for estimating casein which is used in the investigation of peptic and tryptic processes by fermentation This method is based on the turbidity produced by guanidine in solutions of casein The investigation of the phenomena and processes produced by diastatic ferment have also been facilitated by the employment of nephelometric methods P Rona and Van Eweyk¹⁶ have described a nephelometric method for the investigation of the action of amylase on glycogen

Thus nephelometry though still in the initial stage of its development has already proved eminently useful in microchemic and biologic research It is, however, all the more important to be careful not to get an intrinsically valuable analytic method into disrepute by employing it injudiciously and indiscriminately It is especially in physiologic chemistry, where substances have often to be subjected to analysis in extremely small quantities, that nephelometric methods promise to be of the greatest importance and advantage On the other hand, nephelometric methods may, owing to the high precision of the instrument, be used with advantage in purely colloidal work For from

Ryleigh's formula it follows that both the volume and the number of the smallest particles of a colloid in solution are proportional to the intensity of the light they emit and, the strength of the solution being known, may therefore be subjected to measurement.

Generally speaking changes in the colloidal state may be detected and gauged by variations in the dispersive power and consequently in the intensity of the light emitted.

REFERENCES

- ¹Kleinmann H Biochem Ztschr 1919 xcix 115 149
- ²Richards T W and Wells R C Am Chem Jour 1904 xxxi 231
- ³Richards T W Am Chem Jour 1906 xxxv 510
- ⁴Kober P A Jour Biol Chem 1912 13 viii 485
- ⁵Bloor W N Jour of Biol Chem 1915 xvi 143 and 1915 xvi 145 Compare also
Mills E B Jour of Biol Chem 1918 xxxvi 335
- ⁶Kleinmann H Kolloid Ztschr, 1920, xxvii 236 compare also the same author in Abderhalden Handb der Biol Arbeitsmethoden (Berlin 1923)
- ⁷Lednicky Kolloid Ztschr 1923 xxxvi 12 17
- ⁸Rusznysak, S Biochem Ztschr 1922 cxxxiii 36 p 361
- ⁹Bloor W N Jour Biol Chem 1914 xvi 371
- ¹⁰Blix Gunnar Skan Arch f Physiol 1921 lvi 121
- ¹¹Bing, H J and Heckscher H Biochem Ztschr 1924 cxlv 79 and 8
- ¹²Rona, P and Kleinmann H Biochem Ztschr cxxxvii 13 p 131
- ¹³Rona, P and Kleinmann, H Biochem Ztschr cxl 46 p 461
- ¹⁴Rona P and Kleinmann H Biochem Ztschr cxl 46 p 478
- ¹⁵Rona, P, and Kleinmann H Biochem Ztschr cxl 4
- ¹⁶Rona, P and Van Eweyk Biochem Ztschr 1924 cxl 175
- ¹⁷Compare Ostwald Wo Light and Colors in Colloids (Dresden 1924)
- ¹⁸Weinberg Biochem Ztschr 1921 cxvi 292
- ¹⁹Compare also Kleinmann H Biochem Ztschr cxxxvii 1 144
- ²⁰Bechhold, H, and Hebler P Kolloid Ztschr xvi 132 137

DIFFUSE CORTICAL SCLEROSIS*

A CLINICAL AND PATHOLOGIC REPORT OF TWO CASES

BY GLANVILLE Y RUSK, M.D., AND CHARLES E NIXON, M.D.
SAN FRANCISCO, CALIF

IN MARKED contrast to those types of cortical sclerosis plainly secondary in character and associated with vascular changes or inflammatory reactions, or to tuberous sclerosis or to the patchy multiple sclerosis there is occasionally found a diffuse cortical atrophy and sclerosis of quite unknown etiology in which there is a uniform atrophy with destruction of the cortical nerve cells and marked overgrowth of the glial tissue. This type of sclerosis is commonly found in children and appears to represent a disease entity both clinically and anatomically.

We have had an opportunity to examine two such cases. For the clinical data and material of the first case we are indebted to Dr L Emmett Holt and to the former resident physician of the Babies' Hospital, New York, Dr Dorothy M Reed, the second case is from the Children's Hospital of San Francisco and the clinical record was kindly given to us by Dr Edith Bionson.

Under the title of "diffuse sclerosis of the brain" one finds included almost every condition showing a glia proliferation,—cases of dementia paralytica, sclerosis due to arteriosclerosis, hydrocephalus with secondary atrophy and sclerosis, tuberous sclerosis, multiple sclerosis, amaurotic family idiocy and various conditions appearing in the literature as meningoencephalitis. A generalized atrophy of the cerebral cortex in children whose history gives no lead as to etiology is evidently a rare condition, Ziehen¹ makes the statement that outside of the motor cortex and occasionally of the speech and sensory areas he cannot report any cases of general cortical dysplasia.

Oppenheim² mentions that diffuse sclerosis occurs in various conditions and with different pathogenesis and nosologic significance. Heubner³ apparently gave the first description of diffuse cerebral sclerosis in children, the child died at the age of five years, in this case there is a question of the etiologic relationship as the child was normal, except for deficient speech development, until he had a head injury at the age of three and three fourths years, on section it was noted that the brain cut like fresh Swiss cheese. The author looks upon the condition as not congenital but developing in a previously healthy individual.

Schmaus⁴ reports the findings in a three-year-old child who had been well until one and three-fourths years old, the brain, especially the convolutions, were markedly atrophied. In this case, however, the child had muscular cramps and fever at the onset and a pachymeningitis interna was

*From the Department of Pathology University of California Medical School and Hospitals

Received for publication Nov. 29 1926

found, the pathologic changes were therefore evidently the result of some inflammatory process

In the condition described by Strumpell⁵ there was an overgrowth and shrinking of the neuroglia, the ganglia cells were apparently normal, the cerebrum was one third of the normal size and the cerebellum of nearly normal size. Since a general perivascular infiltration was found the process was possibly a chronic encephalitis.

Under the title of "Diffuse Cortical Sclerosis of the Brain in Children" Bullard⁶ reports a case and discusses the relationship of this condition to head injuries. His patient was a boy aged thirteen years. He regarded the hydrocephalus as secondary to a diffuse sclerosis. He says "Schmaus' case is somewhat analogous," but thinks it should be classed among the lobar scleroses which may be very diffuse.

Marchand and Nouet⁷ state that the pathologic picture they term "la sclerose cerebrale superficielle diffuse" may result from a chronic meningitis due to various intoxications or infections. It may be an anomaly in development of the brain, a defect in equilibrium existing from birth between the nerve cell elements and the neuroglia tissue. It may develop in a previously normal brain under the influence of a toxin or infection without concomitant alteration of the meninges or cortical vessels. He noted an alteration of the tangential fibers.

Krabbe⁸ describes a "new familial infantile form of diffuse brain sclerosis" in which the changes are almost entirely in the white matter. There is a replacement of the destroyed tissue by neuroglia with relative intactness of the nerve cells.

Two cases of diffuse cerebral sclerosis were reported by Moser,⁹ one a nine year old boy and the second a twelve year old girl. In these cases the histologic examination indicates an inflammatory alteration in the sense of Schmaus.

REPORT OF CASES

CASE 1.—The patient was a female child aged two years and ten months at the time of its admission to the Babies Hospital. Its hereditary and familial history is quite negative and unsuggestive. Birth was at full term with normal delivery. There was no evidence of lues. The child had developed normally up to its present illness. It was nursed for four months, then fed on diluted cow's milk, and since one year of age had taken table food. The child cut its teeth beginning at one year, walked well at eighteen months, and talked words. There is no history of contagious diseases or gastrointestinal disorder.

The present illness began insiduously when the child was somewhat less than two years of age. At first it was noticed to stumble and fall. The condition gradually increased so that after about four months it could not walk or stand, it could, however, still creep. For six months previous to admission the child had been helpless, not being able to use its feet at all and later becoming uncertain in the use of its hands. Speech and understanding went in the same gradual manner until the child became "idiotic" as well as helpless and showed impairment of hearing and sight. For six months previous to the hospital entrance there had been constant movements of the hands and feet. No definite convulsions were noted but the child would jerk suddenly and stiffen out. There was no fever, vomiting or headache, while under observation there was no attempt at sphincter control.

the pia. In the molecular layer the glial cells are increased in size and are rich in fiber formation, but beneath this zone, scattered diffusely, are large protoplasmic rich, often binucleated neuroglia cells with a remarkable wealth of processes which are margined by, and through the centers of which run, numerous fibrillae giving the characteristic reaction of neuroglial fibrils (Figs 3 and 4). The processes may frequently be traced to vessels about which they twine forming a loose meshlike appearance which probably is largely an artefact and which corresponds to the line of tearing noted in the gross description.

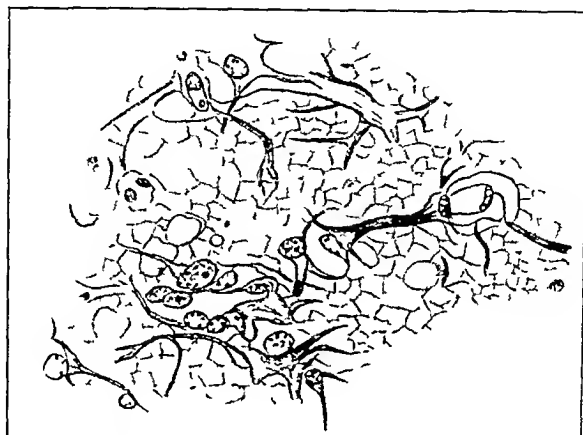


Fig 3—Neuroglia—phosphotungstic acid hematoxylin. (Case 1)



Fig 4—Neuroglia—phosphotungstic acid hematoxylin. (Case 1)

Considering the anchoring of the pia to the cortex by the superficial glia and similarity of the cortex to a layer of raw cotton in which nerve cells lie embedded, an artefact of this type may be explained. It is especially noticeable that very few of the small naked satellite glial cells are found, all having undergone the hypertrophy. With all the glial hypertrophy no mitotic figures were encountered.

In the immediate subcortex again the glia is hypertrophic though less conspicuously so. It is unfortunate that Mallory's phosphotungstic acid hematoxylin stains the myelin, making search in such regions quite difficult, but in the modified Nissl stain evidence of

similar hypertrophy is distinct. The degree gradually diminished but it is present generally in the basal ganglia and thalamus. In these situations the hypertrophic glia often appear as long, thin cells with tapering ends either curved or straight, angular or branched, the nucleus is centrally situated long and narrow with rounded ends and contains a few chromatin particles. These correspond to the rod cells of Nissl which are of not unusual occurrence in general paralysis of the insane and less frequently in senile dementia. In this region many small, deeply stained glial nuclei of the usual type are seen in contrast to the cortical findings.

The glia of the cerebellar cortex is hypertrophied, Bergman fibers being irregularly seen as thick branches running through the molecular layer. The general picture, however, does not approach the severity of the condition found in the cerebral cortex.

The neuroglia of the spinal cord does not show any definite hypertrophy, even in the region of the crossed pyramidal tracts which as we shall see are diffusely thinned, the reaction fails.

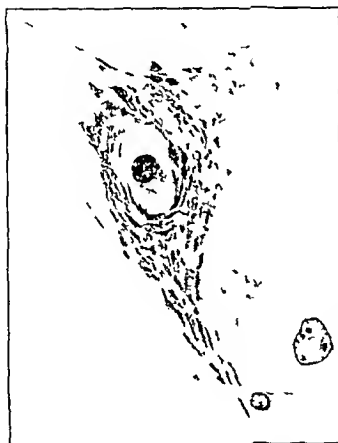


Fig 5—Cortical ganglion cell (Case 1)

On the part of the nerve cells marked changes are found. The abnormality is of one type but varies in degree depending to a considerable extent on the size of the cell. The nervous elements of the whole cerebro spinal axis are affected and a morphologically normal cell is rarely seen.

One may best obtain an idea of the abnormal changes in the cells by observing one of the larger cell types, an anterior horn cell of the spinal cord or a large motor cell from the paracentral region. Primarily the change may affect the portion of the cell from which the axis cylinder arises, and consists of a breaking up of the Nissl bodies giving a diffuse dusty appearance to this portion of the cell. The change is accompanied by a local swelling of the cytoplasm (Fig 5).

Few cells show so early a change but most have proceeded to a stage where the Nissl bodies have disappeared from the swollen portion of the cell leaving a finely reticulated or perhaps vacuolated appearance locally, in which there is a yellowish cast to the cytoplasm strongly suggesting a prepigment stage. The swollen portion of the cell varies in amount within wide limits. In the remainder of the cell the Nissl bodies are well preserved and extend normally along the dendritic processes.

DIFFUSE CORTICAL SCLEROSIS*

A CLINICAL AND PATHOLOGIC REPORT OF TWO CASES

BY GLANVILLE Y. RUSK, M.D., AND CHARLES E. NIXON, M.D.
SAN FRANCISCO, CALIF.

IN MARKED contrast to those types of cortical sclerosis plainly secondary in character and associated with vascular changes or inflammatory reactions, or to tuberous sclerosis or to the patchy multiple sclerosis there is occasionally found a diffuse cortical atrophy and sclerosis of quite unknown etiology in which there is a uniform atrophy with destruction of the cortical nerve cells and marked overgrowth of the glial tissue. This type of sclerosis is commonly found in children and appears to represent a disease entity both clinically and anatomically.

We have had an opportunity to examine two such cases. For the clinical data and material of the first case we are indebted to Dr. L. Emmett Holt and to the former resident physician of the Babies' Hospital, New York. Dr. Dorothy M. Reed, the second case is from the Children's Hospital of San Francisco and the clinical record was kindly given to us by Dr. Edith Bronson.

Under the title of "diffuse sclerosis of the brain" one finds included almost every condition showing a glia proliferation,—cases of dementia paralytica, sclerosis due to arteriosclerosis, hydrocephalus with secondary atrophy and sclerosis, tuberous sclerosis, multiple sclerosis, amaurotic family idiocy and various conditions appearing in the literature as meningoencephalitis. A generalized atrophy of the cerebral cortex in children whose history gives no lead as to etiology is evidently a rare condition. Ziehen¹ makes the statement that outside of the motor cortex and occasionally of the speech and sensory areas he cannot report any cases of general cortical dysplasia.

Oppenheim² mentions that diffuse sclerosis occurs in various conditions and with different pathogenesis and nosologic significance. Heubner³ apparently gave the first description of diffuse cerebral sclerosis in children; the child died at the age of five years, in this case there is a question of the etiologic relationship as the child was normal, except for deficient speech development, until he had a head injury at the age of three and three-fourths years, on section it was noted that the brain cut like fresh Swiss cheese. The author looks upon the condition as not congenital but developing in a previously healthy individual.

Schmaus⁴ reports the findings in a three-year-old child who had been well until one and three-fourths years old, the brain, especially the convolutions, were markedly atrophied. In this case, however, the child had muscular cramps and fever at the onset and a pachymeningitis interna was

*From the Department of Pathology, University of California Medical School and Hospitals.

Received for publication Nov. 29, 1926.

found, the pathologic changes were therefore evidently the result of some inflammatory process

In the condition described by Stumpell⁵ there was an overgrowth and shrinking of the neuroglia, the ganglia cells were apparently normal, the cerebrum was one third of the normal size and the cerebellum of nearly normal size. Since a general perivascular infiltration was found the process was possibly a chronic encephalitis.

Under the title of 'Diffuse Cortical Sclerosis of the Brain in Children' Bullard⁶ reports a case and discusses the relationship of this condition to head injuries. His patient was a boy aged thirteen years. He regarded the hydrocephalus as secondary to a diffuse sclerosis. He says 'Schmaus' case is somewhat analogous,' but thinks it should be classed among the lobar scleroses which may be very diffuse."

Marchand and Nouet⁷ state that the pathologic picture they term 'la sclerose cerebrale superficielle diffuse' may result from a chronic meningitis due to various intoxications or infections. It may be an anomaly in development of the brain, a defect in equilibrium existing from birth between the nerve cell elements and the neuroglia tissue. It may develop in a previously normal brain under the influence of a toxin or infection without concomitant alteration of the meninges or cortical vessels. He noted an alteration of the tangential fibers.

Krabbe⁸ describes a "new familial infantile form of diffuse brain sclerosis" in which the changes are almost entirely in the white matter. There is a replacement of the destroyed tissue by neuroglia with relative intactness of the nerve cells.

Two cases of diffuse cerebral sclerosis were reported by Moser,⁹ one a nine year old boy and the second a twelve year old girl. In these cases the histologic examination indicates an inflammatory alteration in the sense of Schmaus.

REPORT OF CASES

CASE 1.—The patient was a female child aged two years and ten months at the time of its admission to the Babies Hospital. Its hereditary and familial history is quite negative and unsuggestive. Birth was at full term with normal delivery. There was no evidence of lues. The child had developed normally up to its present illness, it was nursed for four months, then fed on diluted cow's milk, and since one year of age had taken table food. The child cut its teeth beginning at one year, walked well at eighteen months and talked words. There is no history of contagious diseases or gastrointestinal disorder.

The present illness began insiduously when the child was somewhat less than two years of age. At first it was noticed to stumble and fall, the condition gradually increasing, so that after about four months it could not walk or stand, it could, however, still creep. For six months previous to admission the child had been helpless, not being able to use its feet at all and later becoming uncertain in the use of its hands. Speech and understanding went in the same gradual manner until the child became "idiotic" as well as helpless and showed impairment of hearing and sight. For six months previous to the hospital entrance there had been constant movements of the hands and feet. No definite convulsions were noted but the child would jerk suddenly and stiffen out. There was no fever, vomiting or headache, while under observation there was no attempt at sphincter control.

Physical examination by Dr Holt, "Stout, well nourished child, good color, no wasting. Almost constant movements of hands, arms and fingers of a general athetoid character, somewhat resembling choreiform movements. Lower extremities are not paralyzed and movement of legs are somewhat similar to those of arms. At times a well marked tremor of feet and toes. Slight ankle clonus. Knee jerks very much increased and there is rigidity more than relaxation. No actual or apparent atrophy. Eyes. Pupils normal, reactive to light. There is slight obliquity of the head and flattening over left temple. Skull otherwise negative. Slight internal strabismus. Normal teeth and gums, clean tongue."

"Mentally. Like child in active delirium. Marked symptoms of cerebral irritation, grinding teeth, rolling head, no rigidity of neck, sees and follows objects. Hearing is doubtful. Symptoms everywhere bilateral. No separation of sutures of skull. Pulse varies from 120-80, when she is restless or quiet. Lies quietly asleep for a few minutes then starts suddenly as if electrified."

The fundi were examined on two occasions by Dr Marple who reported incomplete atrophy of both discs. No cherry red spot observed.

Antisiphilitic treatment was begun but without results. Lumbar puncture yielded a clear culturally negative fluid. No cytologic examination was made.

The neurologic progress of the case (slightly less than two months till death) was marked by periods of apathy alternating with states of wild excitement, with incoordinated bizarre movements involving all the extremities, especially the arms and to a less extent the trunk. The excited periods were at times precipitated by disturbing the child and were more marked during the infections from which the child suffered towards the end. Later the movements took on a rhythmic character, varying from four to seventy per minute. About a week before death the child lay on its back, arms extended at the sides, thighs abducted and knees flexed on thorax, legs flexed on thighs, very little rigidity, no Kernig's sign. Knee jerks not obtained, quite marked foot drop. No ankle clonus, no Babinski's sign. No opisthotonos. Tache cerebrale well marked.

A series of infections supervened. A tonsillitis, later a double purulent otitis media which ruptured, and finally terminal bronchopneumonia. During the febrile periods, erythematous rashes appeared, they were more or less transitory and occurred on the chest and abdomen. Periods of pallor or cyanosis, likewise transitory, occurred. Respiration towards the end became irregular but not of Cheyne Stokes' type. Feeding was by gavage and well retained. Irregular vasomotor disturbances about face and neck were noted late in the disease and the rash over the chest and abdomen towards the end assumed a macular, punctate form character lasting twenty four to forty eight hours, and then completely disappearing. Death with bronchopneumonia and general inanition.

The diagnosis of the neurologic condition was in doubt. Dr Pierce Bailey suggested a neurotic family idiosyncrasy, and one of us (G. Y. R.) who saw the case on one occasion through the courtesy of Dr Reed, was impressed with the marked ataxic character of the movement and taken together with the optic atrophy, suggested a tumor involving the cerebellar apparatus. Neither, however, was substantiated by the anatomic findings.

The necropsy was performed by Dr Martha Wolstein. The brain together with portion of the cervical cord was fixed in formalin.

The fixed brain presented a symmetrically and diffusely thick, grayish, boggy, leathery arachnoid. This membrane is so thick that no adequate idea of the fissuration is obtainable while it is in place. In removing the pia it is found to be tough and the stripping shows a distinct tendency to tear the surface of the cortex, not causing small punctate defects but distinct and extensive defects of tissue. Roughly one third to one half the cortex appeared to come away. The cortex superficially is distinctly soft and spongy throughout, with little variation over the whole surface, except that the condition was especially noticeable over the central convolutions and in the calcarine areas. The pia being partly stripped, the underlying cortex showed a high grade of diffuse symmetrical atrophy, slightly more accentuated frontally. The sulci are wide, yet shallow, and the convolutions shrunken, soft to the touch superficially, but on deep palpation there is an increased elastic firmness to the touch (Figs 1 and 2). This is particularly brought out on sectioning, the tissue cutting as

rubber were incorporated with it. There is no abnormality of convolutional distribution. No patches of discoloration or evidence of focal lesion of any type were encountered. The cerebellum was symmetrical, the folia somewhat shrunken, the pia not nearly so gray and thickened as that of the cerebrum, though here also somewhat adherent. The vessels at the base were normal in appearance and distribution. The floor of the fourth ventricle showed no granulations. The cranial nerves appeared normal throughout, except the optic nerve which seemed somewhat small but did not show the grayness of atrophy of high degree.

The microscopic examination of the material brought out a remarkable diffuse neuroglial hyperplasia occurring throughout the cortex and to a much less extent in the white matter of the brain and central nuclei. Secondly, a peculiar alteration of the nerve cells

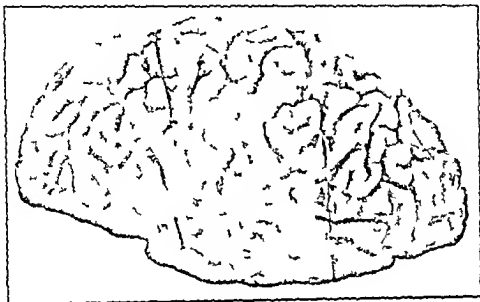


FIG. 1

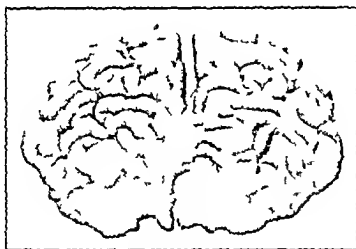


FIG. 2

Figs. 1 and 2.—Photographs of brain of Case 1

not only of the cortex but involving the nerve cells generally throughout the central nervous system, in the third place an absence of tract degeneration except some thinning of both crossed pyramidal tracts, fourthly, an absence of inflammatory elements in the histologic picture and lastly, an hypertrophy of the pia probably entirely compensatory in nature.

The principal or at least the most striking feature of the microscopic picture is the glial hyperplasia diffusely affecting the whole cortex; no part escapes even where the cortex is rolled up to form the fascia dentata, glial hypertrophy occurs. In fact the process is so diffuse and evenly distributed that it would be more than superfluous to describe the various small sections in detail. Sections stained for neuroglia by Mallory's phosphotungstic hematoxylin, show the superficial layer of glia as a more or less thickened felt work from which here and there bunch like masses of fibers entwine and mingle with the connective tissue of

the pia. In the molecular layer the glial cells are increased in size and are rich in fiber formation, but beneath this zone, scattered diffusely, are large protoplasmic rich, often bi or tri nucleated neuroglia cells with a remarkable wealth of processes which are margined by, and through the centers of which run, numerous fibrillae giving the characteristic reaction of neuroglial fibrils (Figs 3 and 4). The processes may frequently be traced to vessels about which they twine forming a loose mesh like appearance which probably is largely an artefact and which corresponds to the line of tearing noted in the gross description.

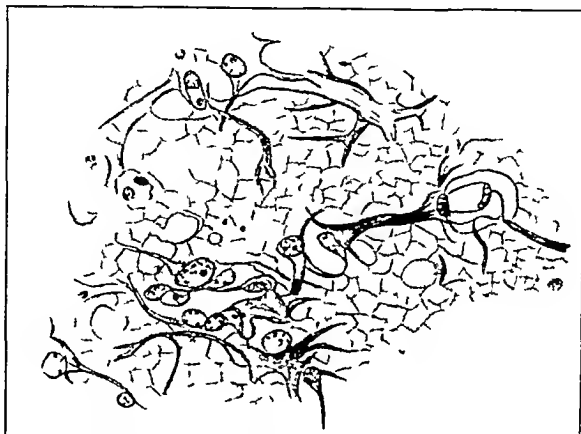


Fig 3—Neuroglia—phosphotungstic acid hematoxylin (Case 1)



Fig 4—Neuroglia—phosphotungstic acid hematoxylin (Case 1)

Considering the anchoring of the pia to the cortex by the superficial glia and similarity of the cortex to a layer of raw cotton in which nerve cells lie embedded, an artefact of this type may be explained. It is especially noticeable that very few of the small naked satellite glial cells are found, all having undergone the hypertrophy. With all the glial hypertrophy no mitotic figures were encountered.

In the immediate subcortex again the glia is hypertrophic though less conspicuously so. It is unfortunate that Mallory's phosphotungstic acid hematoxylin stains the myelin, making search in such regions quite difficult, but in the modified Nissl stain evidence of

similar hypertrophy is distinct. The degree gradually diminished but it is present generally in the basal ganglia and thalamus. In these situations the hypertrophic glia often appear as long, thin cells with tapering ends either curved or straight angular or branched. The nucleus is centrally situated, long and narrow with rounded ends, and contains a few chromatin particles. These correspond to the rod cells of Nissl which are of not unusual occurrence in general paralysis of the insane and less frequently in senile dementia. In this region many small, deeply stained glial nuclei of the usual type are seen in contrast to the cortical findings.

The glia of the cerebellar cortex is hypertrophied. Bergmann fibers being irregularly seen as thick branches running through the molecular layer. The general picture however does not approach the severity of the condition found in the cerebral cortex.

The neuroglia of the spinal cord does not show any definite hypertrophy even in the region of the crossed pyramidal tracts which as we shall see are diffusely thinned. The reaction fails.



Fig. —Cortical ganglion cell (Case 1)

On the part of the nerve cells marked changes are found. The abnormality is of one type but varies in degree depending to a considerable extent on the size of the cell. The nervous elements of the whole cerebro spinal axis are affected and a morphologically normal cell is rarely seen.

One may best obtain an idea of the abnormal changes in the cells by observing one of the larger cell types an anterior horn cell of the spinal cord or a large motor cell from the paracentral region. Primarily the changes may affect the portion of the cell from which the axis cylinder arises and consists of a breaking up of the Nissl bodies giving a diffuse dusty appearance to this portion of the cell. The change is accompanied by a local swelling of the cytoplasm (Fig. 5).

Few cells show so early a change but most have proceeded to a stage where the Nissl bodies have disappeared from the swollen portion of the cell leaving a finely reticulated or perhaps vacuolated appearance locally in which there is a yellowish cast to the cytoplasm strongly suggesting a prepigment stage. The swollen portion of the cell varies in amount within wide limits. In the remainder of the cell the Nissl bodies are well preserved and extend normally along the dendritic processes.

The nucleus in the larger cells appears hyperchromatic and as a rule is situated in that portion of the cell which contains the Nissl bodies, it is often surrounded by an especially marked layer of Nissl substance which may obscure the nuclear membrane. As the size of the cell diminishes the relative size of the area of swollen, Nissl body free cytoplasm increases, this statement holds particularly true for the cortex where in the small pyramidal cells we have an extreme example of the end stages of the process. There, what were formerly small pyramidal cells are swollen, bulbous or nearly circular bodies with all trace of chromatic substance wanting, the cytoplasm of a yellowish tinge and reticulated. The nucleus undergoes a progressive diminution in size, appearing not as a circular body but as a little irregular dot, sharply circumscribed body with no internal differentiation, lying usually against the cell wall. As described above the size of the cell rather than the position seems to predicate the degree of the reaction, but also there seems to be some relation to the degree of glial hypertrophy and the stage of change reached by the nervous elements, most marked in the cortex and less so in the basal ganglia where the cells do not reach the more extreme grade of reaction. The central nucleus of the thalamus for example, shows very uniformly its cells shaped like acorns, with bulbous bases of reticulated cyto-



Fig 6—Section of cerebral hemisphere stained by Weigert's myelin sheath method (C. 1)

plasm, and the nuclei diffusely hyperchromatic, compressed into the beginning of the apical processes and closely surrounded by a small quantity of dense chromatic material. Equivalent results would follow description of cells from other regions, the cerebellum, the medulla, or any of the many cortical regions observed. It might be stated that as a group probably the cells of the posterior olfactory regions and the dentate fascia show less change than those of any other functional system.

We now come to the picture presented in the tissues stained by Weigert's myelin sheath method (Fig 6). Surprisingly little abnormal is revealed by it. In the cortex the tangential fibers are generally present though separated more than normally, apparently by the great glial hypertrophy. The radial zone is less myelin rich than the normal adult cortex, but in the white substance, no degenerative change is of sufficient extent to make itself visible. Especially to be noted is the intactness of the optic nerves and tract, showing that what there was of visual dimness must either have been due to retinal or cortical disturbances. This cortex shares the fate of all the remainder and to a marked degree the retinae were unfortunately not available for examination. In the spinal cord, however, there is a diffuse though slight thinning of the crossed pyramidal tracts. This may represent a progressive retrograde degeneration of some of the long fibers beginning distant from their cells.

As to the mesodermal elements little can be said. The thick boggy pia shows simply an increase of the connective tissue elements and nothing of a chronic inflammatory character. There is, however, a rather striking pigmentation of the leptomeninges consisting of a golden yellow pigment contained in phagocytes as well as similar pigment apparently lying free. The vessels also are negative, both in the pia and in the cortex. There is no hypervascularity. The perivascular spaces are prominent and the relation of the hypertrophied glia to them has already been noted.

CASE 2—Clinical History by Dr. L. Bronson. E. C., male, aged ten months, was admitted to the Children's Hospital March 7, 1921, with the complaint of vomiting and making no effort to sit up.

Family History. Neither father nor mother is strong. A sister, five years old, is healthy. No family history of tuberculosis, syphilis or insanity.

Past History. Full term normal birth though prolonged. Weight six and three fourths pounds. Breast fed without difficulty for two to three months but taken from breast then because of failure to gain. Later feeding was on goat's and cow's milk formulas. The baby was never hungry and took only about one third of the food offered. Vomiting not projectile started soon after the baby was taken from the breast. Milk of magnesia was given daily for constipation.

Mother had noticed that he did not know her from a stranger, that he did not pay attention when she was preparing his bottle, that he cried when she held him and preferred to be left alone. He never smiled or turned his head toward a light or seemed to notice a sound.

Examination. Weight fourteen pounds, six ounces. A much undernourished but not greatly undernourished infant. Color good.

Head. Suture lines palpable. Fontanelles were closed. Hair abundant and long. Head measurements showing general reduction are given below.

Eyes. Pupils react to light but the eyes will not follow a light. Winks when a bright light is suddenly flashed in the face. No strabismus or nystagmus. Eye grounds are negative, no cherry red spot.

Ears. negative. **Tooth.** two lower incisors. **Tonsils.** small lymphoid glands not enlarged. **Lungs.** a generalized bronchitis. **Heart.** negative. **Abdomen.** negative. **Genitalia.** testicles undescended.

Urine. negative.

Blood. Reds 4,000,000, whites 9,800. Differential polymorphonuclear 65, lymphocytes 33, large mononuclear 3. Von Pirquet negative.

Cerebrospinal fluid. 10 c.c. of blood tinged fluid not under pressure. Wassermann negative.

Special Condition. An exceedingly spastic infant showing at times partial relaxation. Legs were not crossed but extended stiff with the toes pointed and arms were held flexed at elbow. At times he went into moderate opisthotonos position against the neck was quite relaxed. The deep reflexes were all much exaggerated. On any attempt to elicit the Babinski or ankle clonus he went into general clonic movements, arms and legs rapidly flexed and extended and very striking clonic movements of the chin. A cephalic cry accompanied these movements when quiet the child always showed spastic extremities yet neck and trunk muscles were often relaxed.

Yawning was characteristic and frequent, a complete yawn, mouth opened, legs extended, elbows flexed. No athetoid movements were noted and there was no ataxia. He screamed frequently, always if touched. It was impossible to test sensation, as handling brought on a general reflex motor reaction.

He was in the Children's Hospital for three weeks. His bronchitis improved. The bowels were loose rather than constipated. He vomited very little. Feeding took much

patience and he gained only five ounces. He had vegetables, cereal, etc., as well as milk, when taken home.

Diagnosis Microcephalus, congenital cerebral aplasia.

When sixteen months old, in August, 1921, he was admitted to the Pediatric Service of the University of California Hospital because of failure to gain. The head measurements were practically unchanged. He had no more teeth. The pupils were equal and reacted to light. The neck had become constantly rigid, and abdominal muscles tense. The

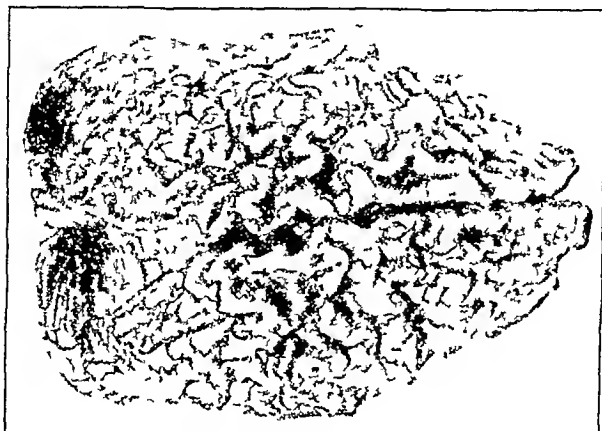


Fig 7

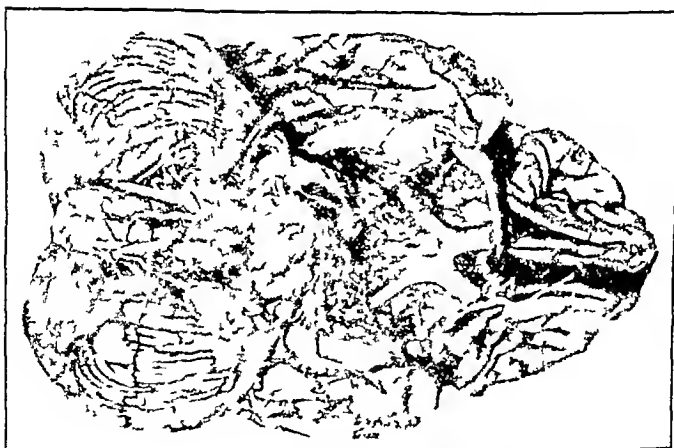


Fig 8

extremities were very spastic except the right leg which was flaccid and atrophic with absent tendon reflexes. According to the nurse's notes, he screamed a great part of the time.

Radiogram of the skull showed general thinning with no distinct suture lines visible. Blood, hemoglobin 51 per cent and red blood count 2,720,000.

Wassermann, negative, both antigens.

Von Pirquet, negative.

Diagnosis microcephalus.

Head measurements were generally diminished as compared to the average normal.

He was in the hospital four days only.

He was brought into Dr. Bronson's office on Oct. 21, 1921, at the age of eighteen months because of wasting and sent again to the Children's Hospital. His weight was two

pounds less than in the preceding March. The examination of the nervous system showed even more striking spasticity than before, the muscles of neck and back standing out like cords with the exception of the right lower extremity. The right leg, thigh, and buttock muscles were atrophied and the leg shorter than the left. The reflex clonus noted on the first admission was no longer present. The clinical picture was that of spastic diplegia with lower motor neuron paralysis of the right lower extremity only. The deep tendon reflexes

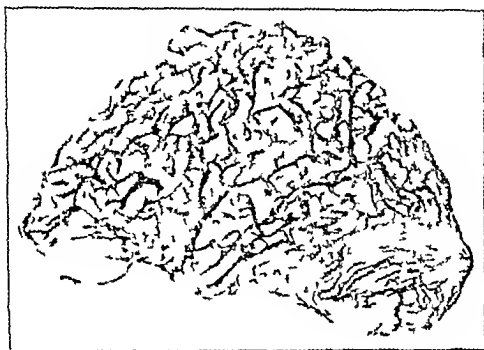


FIG. 9

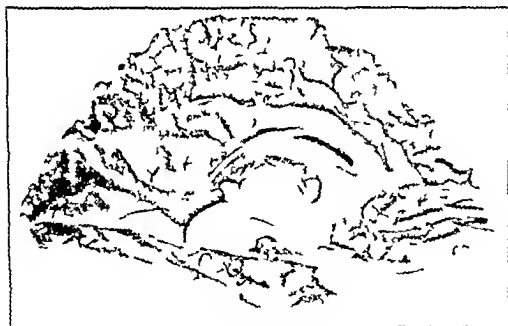


FIG. 10

Figs. 8, 9, 10.—Photographs of brain of Case

were absent on the right. No history of the time of onset of the flaccidity could be obtained. He died apparently of diarrhea.

Clinical observation of this child recalled Sherrington's description of the decerebrate cat. The behavior was distinctly that of the reflex animal. The etiology could not be determined. A pachymeningitis with proliferation of the meningo-vascular tissue and secondary cerebral involvement or a primary aplasia of the cerebral tissue with secondary meningo-vascular proliferation were possibilities. Tay-Sachs disease was ruled out by examination of eye grounds.

In regard to the lower motor neuron paralysis, acute poliomyelitis, localized hemorrhage, or a focus of infection secondary to the upper respiratory infections, was considered.

The autopsy was performed by Dr Rusk and the anatomic diagnosis was as follows. Microcephaly Diffuse atrophy of the cerebrum and the cord External hydrocephalus. Pulmonary tuberculosis Shortening and atrophy of the right leg Undescended testicles

In the detailed report of the nervous system it was noted that "the dura is very firmly adherent to calvarium and when torn away the bone shows reddish areas in the diploe The dura is not thickened but on either side of falx there is a translucent gelatinous pseudomembrane about 3 mm thick most marked on the right side where it shows laminations There is marked external hydrocephalus filling a space about 3 cm between the dura and the atrophied cerebrum There are about 250 cc of fluid which is normal in appearance

The brain weighs 160 gm, the length of the right hemisphere is 10 cm and of the left hemisphere 9.5 cm, the width at the base of the brain is 7.4 cm, the total width of the cerebellum is 7 cm The gross appearance of the cortex of the brain resembled the surface of a pecan nut, the gyri were narrow and the sulci were wide, it is apparent that the convolutions developed normally and no marked pathologic changes occurred until after the convolutions were well formed (Figs 7, 8, 9, 10)

Base of the Brain The vertebral and basilar arteries show no abnormality The circle of Willis is negative except that the internal carotid arteries are smaller than normal The middle cerebral branches are easily seen as they pass through the gaping sulci of the fissure of Sylvius The arterioles are not injected and the veins are not distended The hippocampal gyri and uncus stand out very prominently The gyri orbitales and recti of the frontal lobes show extreme atrophy and are narrowed to 1 cm in front The cerebellum is much larger proportionally than the hemispheres and seems well formed, but the left lobe is larger and slightly darker in color than the right The pons, medulla, basal ganglia and cranial nerves are not remarkable The optic nerves are small The pia is negative

Superior and Lateral Surfaces The right hemisphere is 0.5 cm longer than the left The longitudinal fissure is wide and at the tip of the occipital lobes measures 2.5 cm, at the tips of the frontal lobe it measures 1 cm The vessels and pial are negative The convolutions are perfectly formed but there is a uniform atrophy with widening of the sulci and the entire cerebrum feels like tissue hardened in formalin The central fissure is very prominent and the fissure of Sylvius measures at the surface from 0.5 cm to 1.5 cm in width

The cord is small, firm and shows a depression just anterior to the posterior horn in the right In the lower thoracic and upper lumbar regions there is a hemorrhagic exudate beneath the dura, especially about the nerve roots with slight adhesions between the pia and dura

MICROSCOPIC EXAMINATION

Cerebral Cortex An intense and fairly uniform proliferation of the glial elements is found throughout the cerebral cortex so marked that nerve cells are found with difficulty Many types of glial cells are present varying from the small cells with a faint margin of cytoplasm around the nucleus to enormous protoplasmic glial cells A number of elongated glial cells are to be seen, some of them definitely rod shaped Neuroglial stains show a striking network of glial fibrilla, this network is rather close and in some areas very dense, though sometimes forming sieve like areas Small glial nuclei are fairly numerous in this network of fibrilla

In many areas the processes of the glial cells can be traced to the blood vessels, at times the intertwinning of the fibrilla forms a zone of denser sclerosis around the vessel Along the margin of the cortex and on the ventricular surface glial fibrilla form a closer reticulum than in the deeper parts of the cortex, this zone of marginal sclerosis is narrow in most sections of the cerebrum This profuse overgrowth of neuroglia along the border forms in places a tuft which extends toward and into the pia

Scattered throughout the cerebral cortex are clumps of cells about the size of small glia nuclei, (Fig. 11), there will be 15 to 20 or more of the cells in a group and they are most commonly situated near the margin of the cortex. In some of the cell clusters there is a meshwork of fibrils that resembles an Alzheimer plaque as occurs in Alzheimer's disease.

In some sections of the cortex especially in the occipital region there are wide meshes in the glial network forming a line of lacunae at the junction of the gray and white matter.

There is a striking paucity of nerve cells throughout the cortex. In many sections no nerve cells are to be found and no normal nerve cells are to be seen in sections from the

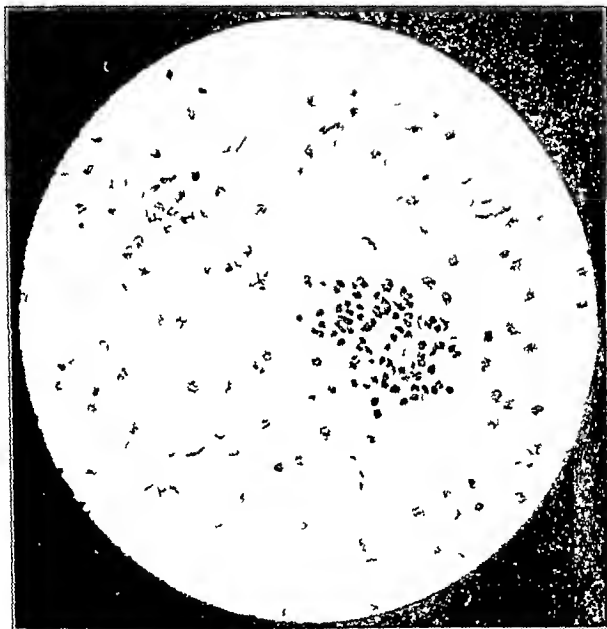


Fig. 11—A clump of glial cells (C. C.)

cerebral cortex. In the most nearly normal cell the nucleus is enlarged and somewhat eccentrically placed, the nuclear substance is somewhat granular and the periphery of the nucleus the chromatin substance of the cell is rather diffusely stained and there is seldom seen the well defined Nissl bodies present in normal nerve cell (Fig. 12.) The axonal portion of the ganglion cells is very faintly stained and in cells showing more advanced chromatolysis no processes at all are to be seen.

In cells where the pathology is only moderate and when the nucleus is always swollen and shows the peripheral arrangement of the nuclear material as a rule the more marked the chromatolytic changes the more eccentrically placed is the nucleus. As the disintegration of the cell progresses it becomes more circular in outline and usually stains rather faintly and diffusely presenting a poorly defined reticulated cytoplasm, occasionally there is well

of raw cotton. The glia tissue is more dense around many of the vessels, and the processes of the glia cells can often be traced to the blood vessel. In both brains there is a formation of lacunae or a line of cleavage in the gray matter but it is much more marked in the first case. In the second case plaques simulating Alzheimers' foci are found throughout the cortex.

The first case shows definite chromatolysis of the cortical nerve cells and a normal appearing cell is rarely seen, in the second brain the cell changes are much more marked, in many sections from the cortex there is not a single nerve cell present and in other sections only cells showing advanced chromatolytic changes or only a "shadow" cell. Satellitosis and the so called neuronphagia are present but nowhere marked. The nerve cells in basal ganglia, brain stem and spinal cord are progressively less involved.

The myelin sheaths of the first brain show little abnormality, the tangential fibers are separated more than normally and there is some irregularity of the myelin sheaths in the radial fibers. In the second case the medullary sheaths show considerable involvement, macroscopically in the Weigert preparations no fibers are seen in the gyri and only a very faint staining in the area just beneath the convolutions, microscopically a few myelin sheaths are seen in the gyri, there is marked irregularity of the sheaths and occasionally there is a row of fat granule cells apparently in the place of a disintegrated nerve fiber.

The meninges are strikingly different in two cases. In the first case there is a thick boggy overgrowth of connective tissue and in the second case there is a thin delicate membrane associated with a marked external hydrocephalus. But they are similar in the absence of inflammatory reactions.

From the standpoint of the pathogenesis of the process it is evident that the gray matter of the cerebral cortex is primarily and most severely involved. It is probable that the condition represents a primary disease of the nerve cells with secondary glial hypertrophy, it is possible, however, that the glial proliferation is primary or the two processes may be intimately associated in their causal relations. There is no evidence of an inflammatory process and the lack of changes in the blood vessels preclude a vascular basis. It is most likely that the disease is an agenic condition.

REFERENCES

- ¹Ziehen in Bruns, Cramer and Ziehen. Handbuch der Nervenkrankheiten im Kinderalter.
- ²Oppenheim. Lehrbuch der Nervenkrankheiten, II.
- ³Heubner, O. Ueber diffuse Hirnsklerose, *Chauve Ann*, 1897, *XXII*, 298-310.
- ⁴Schmaus, H. Zur Kenntniss der diffusen Hirnsklerose, *Virchow's Arch f Path Anat*, 1888, *cxiv*, 154-172.
- ⁵Strumpell, A. Ueber diffuse Hirnsklerose, *Arch f Psychiat*, 1897, *IX*, 268-285.
- ⁶Bullard, Wm N. Diffuse Cortical Sclerosis of the Brain in Children, *Jour Nerv and Ment Dis*, 1890, *xv*, 699-709.
- ⁷Marchand, L. and Nouet, H. Les Syndromes Mentaux Symptomatiques de la Sclérose Cérébrale Superficielle Diffuse, *Rev Neurol*, 1907, *xv*, 1164-1169.
- ⁸Krabbe, Knud. Infantile Form of Diffuse Brain Sclerosis, *Braun*, 1916, *XXIX*, 74-114.
- ⁹Moser, P. Zweier Fälle von diffuser Hirnsklerose, *Mitt d gesellsch f inn Med u Kinderh in Wien*, 1907, *vi*, 79-80.

WELCOME TO DALLAS*

By DR EDWARD F COOKE HOUSTON TEXAS

LAST year, my younger brother, Jack was elected vice president of the Texas Lumberman's Association, at Beaumont. Returning home, full of the honor conferred upon him his train ran into a cow, damaged the cow, derailed the engine and flung Jack from one end of the diner to the other. After he had been gathered out of the debris of chairs and tables and had absorbed the contents of all available flasks he opened his eyes and said, 'If this is a part of the job I resign right now.' If, when I received my program last week, and was aware for the first time of the job assigned me, I had properly visualized this audience of brilliant men and wonderful women I, in the words of my brother, would have resigned right now. I am not nor have I ever been what might be termed a tall man. Normally my height is about five feet, three and a half inches in the morning in my stocking feet but ever since I entered this room I have been shrinking and shrinking until now I can fully enter into all the feelings and sympathize thoroughly with all the reactions of any ultramicroscopic filtrable virus.

As the program and the Toastmaster have informed you I hail from the City of Houston in this glorious State of Texas. I am very proud of my home town. I am glad to say that I can claim without fear of successful contradiction, and prove my claim by our Chamber of Commerce and our local daily newspapers, that Houston is the largest inland port in the world that she is the greatest cotton center in the world that she is the greatest oil center in the world, that she is the greatest lumber center in the world that she is the greatest cattle center in the world that she is the greatest railroad center in the world, and she would be the greatest city in Texas if it wasn't for Dallas. It might not be such a bad idea if some of you went on down to Houston and expressed your unbiased opinions as to whether or not Dallas is a bigger or better city than Houston. This is and has been a matter of much debate pro and con the pro has usually been on Houston's side and the con on the side of Dallas. So we would be very glad of your assistance in settling the matter once for all. One suggestion however. It would perhaps be wiser for you to await your return home before expressing your opinions.

You see this city of Dallas has a most infamous way of looking at the accomplishments of other cities, and then going them one better. San Antonio offers a six thousand dollar purse to the golfers. Dallas watches this for a while and then offers a ten thousand dollar purse. Last year Houston had the Admen's convention, last fall Dallas entertained the Southern Medical Association. Not satisfied with that, she grabbed the American Medical As

Read before the Fifth Annual Convention of the American Society of Clinical Pathologists Dallas Texas April 15, 16 and 17 1916.

sociation, and still not satisfied, she topped that by grabbing the American Society of Clinical Pathologists Galveston has her annual Splash Day, and oh, boy! San Antonio has her Annual Fruit, Flower and Vegetable Carnival, Waco has her annual Cotton Palace, Ft Worth has her annual Live Stock Show All of these are glorious and wonderful, but Dallas has her Annual State Fair, which is the biggest show of all To the best of my knowledge, recollection, and belief, only two events have taken place in the Galveston Houston district that Dallas has not emulated and surpassed. One of these was the hurricane of 1900, and the other was the hurricane of 1915

You recall the old old story of the mountaineer who, in his hospitality, told the visitor, "You're welcome, stranger You're welcome to a potato, take two potatoes, take damn nigh all of them" In the same spirit of hospitality, having canvassed the State pretty thoroughly, on behalf of Galveston, on behalf of Houston, on behalf of San Antonio, Waco, and Ft Worth, especially on behalf of Ft Worth I say to you, you are welcome to Dallas Remember, however, if you accept this offer, it is entirely at your own risk We cannot assume any responsibility, and you must undertake to remove the place from the State entirely After that we do not care what you do with it

Dallas is never satisfied Like the magnet in the Gilbert and Sullivan opera

A magnet hung in a hardware shop
And all around was a glorious crop
Of scissors and needles, knives and forks,
But it wanted a Silver Churn "

You would think as you walk around these busy streets, noting the commerce carried on, looking at the magnificent buildings, reflecting upon all the rich agricultural country surrounding, that Dallas should be a happy contented city But she is not, and the reason is that Dallas yearns to be a port Her reasoning, of course, is perfectly sound and logical Houston is a port and, therefore, Dallas can be a port also There is another rather insufficient reason You may have noticed that Dallas is built on a bluff a big bluff If you stand on the edge of this bluff you can look down upon a little streak of moisture below This streak is the Trinity River, which a little distance south of Dallas becomes quite a stream Sometimes when we have a little rain up this way, this Trinity River gets so that you can see it distinctly with the naked eye Whenever this happens Dallas becomes a dreamer of dreams and a seer of visions, and again discusses the desirability of making the Trinity navigable And do you know, it wouldn't surprise me if they do it some day

But these Dallas folks are such terrible boasters Nothing is small enough to escape their attentions along this line I was enjoying a game of golf one day over one of their beautiful courses I trust that you have had, or will have, an opportunity of agreeing with me that they have some very interesting golf courses here While playing we heard three distinct shots

from either a rifle or revolver. I laughingly said, 'Oh ho! another Dallas woman shooting her husband.' One of the foursome, a Dallas lawyer, was just about to try and make a two foot punt that should have been worth at least fifty cents to him. He turned to me with a look of outraged civic pride and said severely, 'You are mistaken Doctor when a Dallas lady finds it necessary to shoot her husband she only needs one shot.'

They were having a regulation meeting here one day to discuss the question of making the Trinity River navigable and several speakers had spoken about the advantages Dallas possessed and lamented the fact that they were so far from the coast admitting that in this one thing Houston had the advantage over them. If they just were as close to the Gulf of Mexico what an advantage it would be. Finally one gentleman, a visitor to Dallas I think maybe he was from Rochester Minn. got up and said that he was surprised to find out that there was even one thing that Dallas lacked but that this lack could be overcome quite simply. It was just a question of a pipe line to the coast he said and then you gentlemen can have the Gulf of Mexico having your countenance steps by next morning if you can just suck as hard as you can blow. The body was shipped home the next day.

This is not a booster's convention. You are not specially interested in any statistics as to the population of this, that or the other city, the number of miles of paved streets or the amount of business done. If you were I could tell you of the variety of Texas rolling prairie around Dallas, flat alluvial plains of the coastal region, the flat prairies with an elevation of six thousand feet of the Pinhandle, the mountains of West Texas and the forests of East Texas.

I could tell you a wonderful history of the State from the pirates of the Spanish Main to the pirates of the present day. I could tell you tales of the gradual coming of law and order. Of the Ranger Captain who when sent to quell a riot was asked on his arrival if he had been sent by himself alone and his answer 'Well you haven't got but one riot here you?' Of Captain Bill McDonald, of whom the Army Captain said 'He would charge Hell with one bucket of water.' But these things would not particularly interest you. Remember though, as you wander around this city that we are only showing you a sample. Dallas is not the only city we have in the State. We are glad to welcome you to a young and lusty Texas to a city and State that has cast aside its swaddling clothes and sensing something of future destiny is surging upwards and onwards. There has always been a reason why the South has not made the commercial progress of other portions of the United States. There have always been two fears in the minds of those who have looked southward. One of these fears was of terrific heat, the other and more potent fear was of deadly disease. Yellow fever and malaria have been the greatest enemies the South has had to overcome. Today the greatest foe we have to face is the remembrance of these that still lingers in the minds of those who are casting keen eyes to the opportunities and rewards the South has to offer.

It would be quite interesting to chase the malarial phantom through this State. A visitor coming from, let us say, California would enter the State at El Paso. Dr. Waite, there, would assure him that they never have any malaria, but that they have an occasional case at San Antonio. Reaching San Antonio Dr. Stout would tell him that they have no malaria, but Houston has a good deal. Upon his arrival in Houston I would insist that malaria is so rare with us that when we get a case we call in our friends to see the slides, but that Beaumont is a regular hotbed of the disease. Dr. Thomson at Beaumont would sic the visitor on to Orange as a malarial locality, and Dr. Ball at Orange would assure him that he would have to go over the line into Louisiana, and then he would find plenty. So it would go all over the State, and we would find the same thing true in Louisiana. The fact of the matter is that to all intents and purposes yellow fever and malaria are things of the past. Science has triumphed over these diseases, and the glory goes largely to the men of the test tube and microscope. We are glad to have you down here to show you what your efforts are doing for our beloved Southland.

In regard to the other chumera, the heat and indolence of the South, look around you and see the truth. It has been shown that more and better work can be done if all the energy of the body be directed to that end, and none diverted to heat the plant. With the twin bogies of terrific heat and prevalent disease relegated to the dim limbo of forgotten things, the South is about to come into her own industrially. None of us, no matter how optimistic we may be, have a sufficient vision to see the things that are about to come to pass in this State. We ourselves do not have any real conception of our resources, we do not fully realize even the vast area of Texas. One night two of my brothers and I left Houston by train. Each travelled in a different direction, two of us reached our destination by about seven the next morning, the third did not reach his until noon, and he was the only one to touch the Texas border.

The members of the medical profession only dimly, and the commercial and business men do not at all, realize the tremendous bearing on their financial welfare of the busy workers with culture media and pipette in a thousand and one laboratories over this country. These workers themselves have no conception of the impetus that their labors are giving to industry everywhere. Do you see Donald Ross peering into his microscope for weary weeks with sweat blinded eyes, until one day the demonstration of the sporozoites of malaria in the salivary glands of the mosquito? Do you hear the pean of joy swelling from his lips,

"This day relenting God
Has placed within my hand
A wondrous thing, and God
Be praised, at his command"

Do you find here any hint that he (Ross) has placed in the hands of a certain Col. Goigas a wondrous tool that makes it possible to unite two

mighty oceans, each bearing its argosies of World Commerce? Do you find here any suggestion that when his own country should be in due peril, and him across the sea were hastening to the rescue, a certain Surgeon General Gorgas could safely recommend sending thousands of men to training camps in a country where once the Spanish moss on the oak trees was called "Nature's Death flag of Malaria"? Do you see any thought in his mind that he had become an empire builder, worthy to stand with his countryman Cecil Rhodes? No indeed, he was just a tired laboratory worker who had successfully finished his stunt, and what does he see?

'I know this little thing
 A million lives shall save
 O Death where is thy sting
 Thy victory O Grave '

Therefore as the men and women who are so largely responsible for the tremendous strides forward that our country is making we are enthusiastic in welcoming you to the State of Texas and the City of Dallas that you may see with your own eyes what you are accomplishing and to express to you the South's debt of gratitude. You do not look upon yourselves as empire builders, you knights of Stains and Reactions you diligent delvers into Nature's deepest secrets, but yours is this Empire its power, its honor and its glory for ever and ever, world without end. Amen

THE INTEGRATION OF HOSPITAL LABORATORY WORK*

BY PHILIP HILLKOWITZ, M D, DENVER, COLO

ACCORDING to a survey made by the American College of Surgeons there are close to one thousand hospitals in the United States and Canada each having a capacity of one hundred beds or over. All of these institutions, in order to comply with the minimum requirements of hospital standardization, presumably have a clinical laboratory equipped for carrying out the various routine examinations comprised under the term clinical pathology. In accordance with an official interpretation of the College these laboratories should be under the supervision of a competent clinical pathologist.

Inasmuch as over 85 per cent of these hospitals have received the approval of the American College of Surgeons, it would follow that all these clinical laboratories are conducting scientific investigations and helping the clinician in his diagnoses and treatment of disease. How far they approach the ideal desired, how closely the theoretic quantity and quality of proper laboratory supervision coincides with the actual state of affairs is beside the present discussion.

The fact remains that we have on this continent a grand army of laboratory workers with ample equipment capable of being marshalled and directed toward the most useful ends and unfolding undreamed of possibilities in the field of research and discovery.

At the present time the work of the individual clinical pathologist is more or less disjointed having no relation to the output of his fellows. Take the case of the average director of the laboratory in a medium-sized hospital. He exercises, to be sure, a most important and useful function in the conduct of the institution. His work in fact is nowadays indispensable. The routine urine and blood examinations throw a flood of light on the disease process and help the clinician in arriving at the diagnosis. The pathologist's interpretation of tissue findings is of far-reaching import in operative procedures. His presence at staff meetings, at the bedside, and in the operating room has a beneficent and stimulating influence on the internist and surgeon promoting exact and scientific methods in the diagnosis and treatment of disease.

Yet when all is said and done, there is often left the feeling of work half done or uncompleted, of an aching void in solid accomplishment. At the end of the year when the hospital laboratory director takes stock of the results of his twelve months efforts, which by the way is not always done, he merely records the number of the various laboratory examinations performed—so many urinalyses, blood counts, Wassermanns, tissue examinations, etc. They form valuable statistics if properly evaluated, but what happens to them? The figures compiled are buried in the hospital archives. So far as their value

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 15, 16 and 17, 1926.

to the profession or to posterity is concerned they are as though hidden in an Egyptian tomb unless some future excavator will bring them out of their subterranean crypt

The average pathologist is rather isolated from his fellows. Outside of the larger centers he may be the only one in his community and has to rely for stimulus and progress on the literature of this specialty in textbooks and in periodicals that more or less remotely deal with subjects of clinical pathology. In passing it may not be amiss to point out the crying need of a special journal devoted entirely to the practical wants of the garden variety of clinical pathologist. This is said with all due deference to the excellent papers that are found in journals allied to our specialty.

A point of contact with his colleagues is reached through the annual conventions of the American Society of Clinical Pathologists which constitute a source of strong stimulation. One comes away from these meetings full of ambition and high resolve to emulate the noble example of our more gifted conferees who are contributing to the advancement of clinical pathology and the scientific practice of medicine. Once however we are back in our former habitat we lapse into our usual rpaths and revert to the routine of our daily labors.

To but few of us it is vouchsafed to rise above the level of our surroundings, surmount all obstacles and make original investigations or a brilliant discovery. Even were we inclined to do original research we know not where to begin. Bewildered by the multiplicity and complexity of problems to be solved we cannot concentrate on one particular thing. Research often requires cooperation of several talents trained in one or more of the fundamental branches of science which the individual laboratory worker has not mastered. The road to original research is long and arduous requiring patience and perseverance in the unflinching pursuit of the goal.

The individual clinical pathologist therefore does not count for much in opening up new avenues for scientific advancement. But what we are unable to do as individuals we can accomplish in the aggregate. Contemplate the potential possibilities of a thousand workers concentrating their attention on a few problems. If the plan is carefully laid out and each one has a well defined task before him, the solution of any given problem will be easy. In other words, let us apply to hospital laboratory work the same principles that obtain in industry and which have made the United States the richest country in the world. Mass production has brought comforts to the many that were undreamed of in the past.

Mathematics and science being indissolubly linked together I am using the term integration of laboratory work to connote the proper coordination of our scattered efforts into a harmonious whole for the benefit of mankind.

The clinical pathologists throughout the country would gladly embrace the opportunity to contribute to the common good. The spirit of research once engendered may kindle the spark of some latent genius who may be stimulated thereby to independent investigation and discovery.

The idea of utilizing the energy and talent of men engaged in the ap

5 A list of problems are enumerated which lend themselves to such integrated investigations

DISCUSSION

Dr Otto Lowy—It seems to me that Dr Hillkowitz's paper is a beautiful dream which I hope will come true. I believe this is a matter which should be properly taken up at our business meeting tomorrow and discussed and acted upon. I believe there is no association in the country that is so capable and fit to do the work that has been outlined by Dr Hillkowitz. I hope this will be brought up.

TREPONEMATOSIS AS SEEN IN THE RURAL POPULATION OF HAITI *

BY COMMANDER C S BUTLER, (MC) U S NAVY, HAITI, AND LIEUTENANT E
PETERSON (MC) U S NAVY, HAITI

IN THIS paper we are using the term "treponematosiis" to include syphilis and the condition called yaws. We believe that the latter is simply one type of the protean disease, syphilis, and we will adduce evidence to substantiate this belief. Some of those who insist upon duality of viruses in treponematosiis have little patience with those of us who believe that yaws and syphilis are identical.

Doctor Spittel, in his work on yaws, pays his respects to the ingenuity of those who differ in opinion from him and then proceeds to describe a disease which all the masters on the subject of syphilis since the time of Fracastoro put together could not differentiate from lues. The doctor makes his diagnosis on the frambesioma which he thinks is unlike anything else in its appearance and in its pathology. This, by the way, is not a fact, for the typical lesion of yaws is exactly like the condyloma of syphilis both histologically and in its general appearance. The condyloma is, to be sure, more often found on the moist parts while the frambesioma tends to appear on the unapposed areas of skin as well as on the moist parts, a fact observed by Pouppe-Desportes 125 years ago. The dualists, however, do not stop with the frambesioma. They also describe the circinate syphilide in the same kit with yaws and throw in, eventually, all the other skin lesions of syphilis for good measure, so that when one is through trying to dig a clinical picture of the entity yaws, out of any textbook on tropical medicine of the present day, he has a severe headache for his trouble and not much else.

Doctor Hugh Stannus,⁸ in recent numbers of the *Tropical Diseases Bulletin*, gives a wonderful collation of the information contained in papers published on syphilis and yaws during the past few years. One cannot read this, however, without being impressed with two thoughts. First, with the truth of Osler's dictum, "Know syphilis in all its forms and manifestations and all other things clinical will be added unto you", and second, with the wide

*From the Public Health Service of Haiti

Read before the American Society of Clinical Pathologists Dallas Texas April 13 1926
and 17 1926

divergence in conclusions reached when different medical investigators are looking at the same facts. It does not make any difference how good an investigator one happens to be, one nevertheless will often fit the facts to his own preconception of the question at hand. If there was ever a medical question which deserved to be looked at with historical perspective, this question of yaws and syphilis is *that* one. If this historical approach had been used in studying the question in different parts of the world, advocates of duality would not ask the medical profession to believe that in such considerable population as that of Guam or Samoa or anywhere else in the world for that matter, syphilis is absent. Such an error is simply due to the fact that the disease does not in these places present the picture of our preconception. The profession would not be asked to believe that yaws had died out from the negro population of the Southern United States where it had been introduced from Africa times without number and where the pabulum left nothing to be desired to propagate the disease true to form.

If yaws were not syphilis, then the frambesioma should be a most common occurrence in the negro population of the South today. If yaws were not syphilis, then our white people of the South should show the frambesioma, for this distinctive (?) lesion of the disease should breed true in the body of the recipient of the virus, whether that recipient be a Negro, a Malay, or a Caucasian. Two hundred and seventy odd years ago Doctor Thomas Sydenham made the following statement regarding lues venerea (syphilis) and yaws:

The lues venerea was introduced into Europe A.D. 1493 from the West Indies, it being before that time, unknown even by name. Hence the disease is usually considered as endemic to the American colonies. In my mind, however, it is rather referable to the coast of Guinea or to some portion of the Negro country thereabout. Thus I think because many of my countrymen have told me that in slave ships, even before they have reached America, the disease breaks out, also that it breaks out with the natives in the country itself, and that independent of any previous unclean intercourse. Indeed in some cases it afflicts a whole family—men, women, and children. The disease that thus comes spontaneously is in no respect different from the true venereal lues. The symptoms, the pain and the ulcers are the same—making allowance for the difference of climate only. The name, however, is different, the African disease is called the yaws.

With all the attempts at clinical and laboratory differentiation which have been made since Sydenham's time, his observations have never been disproved. It should have been the duty of those who advocate duality to disprove Sydenham's statements rather than to maneuver those who believe in unity into the defensive position of having to prove that yaws is something other than syphilis. As each item in the more exact knowledge of syphilis is unfolding itself, it is very quickly found that the same fact is true for so-called yaws.

We shall only mention a few of these:

- 1 The *Treponema* of so-called yaws was found to be identical with *T. pallidum*.
- 2 The serum reactions were found to be exactly parallel.
- 3 The clinical course of yaws is identical with that of syphilis.
- 4 The specifics of yaws are the same as those of syphilis.

5 The histopathology of the so-called yaws is identical with that of syphilis when the *Treponema* is alone in the lesion in question

One of the writers of this paper (C S B) has answered most of the minor objections brought up by the dualists. Reference in this connection is made to the following papers published upon this subject^{1, 2, 3}

In trying to build a clinical entity out of yaws it is always necessary to bear in mind the marked peculiarities which syphilis shows when operating upon a native race which race does not treat the condition. Failure to remember these peculiarities is the rock upon which many "maiden voyages" in tropical syphilology have come to grief. The man who knows his native syphilis loses all interest in one of the learned dissertations upon native rural syphilis which tries to make it produce aneurysms of the aorta, general paresis of the insane, tabes dorsalis, and, closer in to the primary, mucous patches and a high percentage of *demonstrable* roseolas and papulo squamous syphilides. On the other hand the worse types of dermatitides, palmar and plantar lesions, and pustular skin lesions are the more common. Again the man who knows his tropical rural syphilis discounts immediately any paper in which the writer swallows the fallacy that in any human community on the earth syphilis is nonexistent. From an extended observation of many good medical men in action here in Haiti and elsewhere in the tropics we know that often they steer their mental ship into this narrow culdesac from which they are rarely able to put about and make for the open sea again. Why native tropical syphilis acts in this peculiar manner we do not know,—it just does. The question of strains of *treponemas* must be thrown out at once as the explanation. This little island of Haiti, not larger than the State of South Carolina, has been swapping *treponemas* with every race of man on the face of the earth for 434 years now and the Haitian melting pot has been able to take all "strains" and convert them into a type which gives the same symptomatic expression in the native Haitian, as it does in the Malay of Oceania and the Philippines or the Negro of equatorial Africa.

In the same manner the various "strains" of *treponemas* introduced into the Southern United States have been converted into the one type producing the orthodox syphilis. Treatment, clothing, and shoes have done it.

The marked influence of the surroundings upon the *Treponema* is well illustrated by Ramsay's observation in Assam. He found that florid yaws was only common among the plain dwellers in the warm season, in the cold season these people and the hill people at all seasons showed only condyloma like lesions in the warm moist regions of the axilla, between the nates, etc., while with the return of the heat weather, or if the hill dweller came down to the hot plain, the disease again became florid.

The frambesoma itself is a typical example of how a *treponematous* lesion is affected by its environment. The attending foreign flora is responsible for the excrescent appearance of this lesion.

In the same manner external influence, such as trauma and superinfection, causes a plantar syphilide, occurring in an untreated and barefooted native, to take on the familiar appearance of the so called "ciab."

The fact that yaws occurs most often in childhood and that it *appears*

not to be transmitted from parent to child hereditarily is at first sight a serious drawback to the full acceptance of identity of viruses. It is necessary to remember in this connection (a) that under primitive conditions of personal hygiene syphilis is not one of the venereal diseases at all, but constitutes one of the exanthemas of childhood, and (b) that native hereditary syphilis and syphilis contracted early in life (syphilis infantum) not only develop in immunity, but when these individuals grow to sexual maturity their disease is either latent or tertiary. In either case there is much less chance of the disease carrying over to the offspring. D. C. McArthur⁶ shows how unlike hereditary syphilis in the European is the native hereditary syphilis. While we do not agree with all McArthur says by any means we are sure he is right in his contention that there is a great deal we do not know about syphilis hereditaria and that our conception of European hereditary syphilis cannot be taken to represent the condition as it occurs among native populations. (c) Good personal hygiene and especially adequate treatment convert this exanthem of 'stone age' childhood into a venereally acquired disease. If this is not true how are we to explain this epidemiologic monstrosity of a contagium capable of venereal transfer stopping at the outskirts of cities where the pabulum for its growth is greater and where personal contacts are multiplied to the *n*th power? How are we to explain the tropical and racial delimitation of a potentially venereal disease? No other contagious disease acts like this jaws if it *really* is limited to the country districts, and to the tropics, and to the dark races. How are we physicians to square ourselves with our conscience when we use a certain set of diagnostic standards to make a diagnosis of syphilis in a white man and the same features and standards to make a diagnosis of some other disease in a native? To our mind things that are equal to the same thing are equal to each other. (d) Much of the so called jaws is due to late eruptions in hereditary syphilis.

Every textbook on tropical diseases makes the statement that jaws is inoculable upon syphilis and vice versa. This statement is made upon some one's pseudo exact ipse dixit and it has been copied by all writers for years with wonderful naivete and punctilious accuracy. If this were true there would in all 'jaws countries' be the usual rate of *venereal* syphilitic chancre. As a matter of fact it is rare to find primary syphilitic venereal lesions in rural Haiti. This is the experience of thirty odd men who know a primary lesion when they see it. Anyone of the Public Health Officers in the ten Public Health Districts of this Republic will subscribe to this statement. It represents the opinion of medical men who have examined and treated many, many thousands of cases of human treponematosis. Should a few antiquated contrary animal experiments be allowed to break down all this human evidence?

These ancient animal experiments are being revamped, however. During the last few years laboratory animals have been made to behave like human beings even in syphilis. An important discovery is that of Chesney and Kemp⁴ who showed that a true immunity against syphilis develops in experimentally infected rabbits. The stumbling block of previous investigators has been the time element. These authors have shown that if a rabbit is treated with

arsphenamine and cured after the syphilitic infection has persisted for more than three months, the animal is then immune and cannot be reinfected with syphilis

The criterion of cure and of absence of infection after reinoculation was the negative results in transfer of lymph nodes and tissue from various parts of the body

This work must, of course, change our previous conception of the cause of resistance to reinoculation. Latent infection is not the only cause for this resistance, apparently a true immunity exists

It is interesting to follow Nichols' experiments after the work of Chesney and Kemp. Nichols has previously attempted to show that no cross protection exists between yaws and syphilis. When Chesney and Kemp showed that the time element has an important bearing on the production of immunity in syphilis, Nichols repeated his experiments and concluded that the experimental suggestion is that long infection with yaws may partially protect against syphilis, and that a long course of untreated yaws in childhood may produce some true immunity to syphilis. Nichols adds, however, that the experimental protection of syphilis against itself is so much stronger than that of yaws against syphilis that the argument as to the identity of the two diseases fails

In his experiments Nichols injected five rabbits with yaws and after a period of time varying from 142 to 376 days terminated the lesions with a sterilizing dose of arsphenamine. Within less than a month attempts were made to infect these animals with syphilis, two proved to be immune, two became infected with small chancres after a considerable time, and one rabbit had no local lesion but did have an infected gland. In a second series of three rabbits infected with yaws for 93, 225, and 91 days respectively the attempt to infect with syphilis succeeded only in the third rabbit which had had yaws for 91 days. Probably the time element had something to do with this result

Voegtlin and Dyer³ in a recent publication showed that of thirteen rabbits infected with syphilis and cured, none gave positive results after reinoculation with either syphilis or yaws

These authors find it difficult to interpret the negative results of the inoculation of the treated rabbits with yaws. They quote the experiment of Neisser, Baermann and Halberstaedter (1906) in which these men succeeded in inoculating a monkey with yaws fifteen days after the appearance of a syphilitic chancre. Voegtlin and Dyer conclude that their treated rabbits, probably on account of the treatment, had developed a refractory state of inoculation with *T. pertenue* as far as the production of a local lesion is concerned. It seems rather odd to conclude that the treatment has such influence on *pertenue* and that some form of immunity or tissue resistance is responsible for the negative reaction in case of *pallidum*. Nichols (1911) and Kolle (1922) have shown that if reinoculation is performed within forty days both inoculations produce chancres. "In other words, persistent syphilitic infection, after a certain lapse of time, produces a condition of the serotum which prevents the development of another chancre on reinoculation." Why should not Nichols' and Kolle's findings explain the experiment of Neisser and his

coworkers, and the findings of Chesney and Kemp with regard to syphilis in the rabbit explain the results of Voegtlin and Dyer with regard to yaws in the same animal?

The above findings by these various investigators are to our minds conclusive of the unity of viruses. These findings correlate the clinical observations of many investigators but place reports of "double" infections in mass in a very peculiar light.

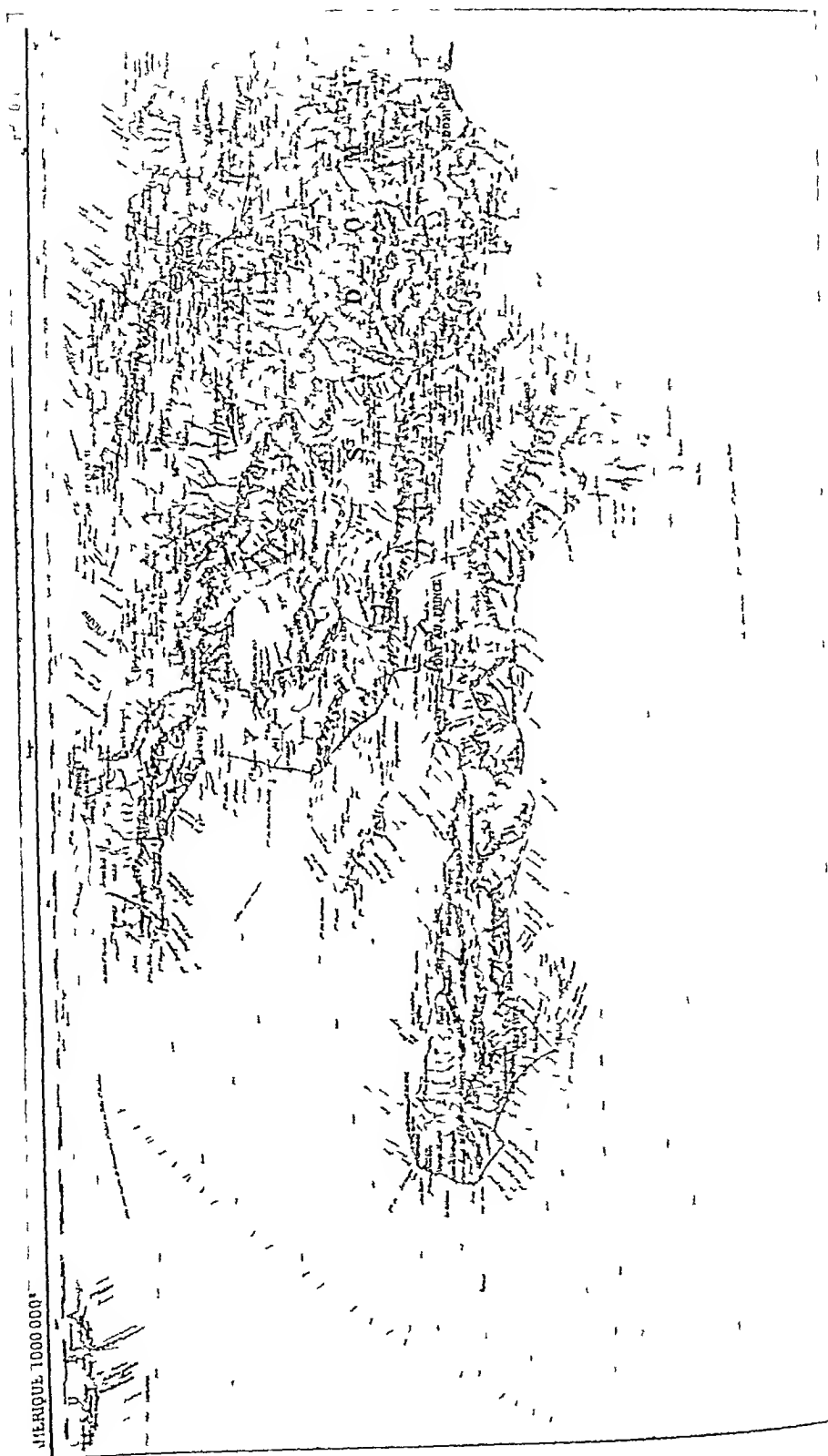
We also believe that the failure of Jahnel and Lange to infect general paralytics with yaws is another link in the chain that ties syphilis and yaws into one, in spite of the fact that Doctor Stannus says that no very definite conclusions can be drawn from these few experiments.

In order to make available the large experience in treponematoses which the Public Health Service of Haiti is accumulating a questionnaire was addressed to three districts located

- 1 In the North (Cape Haitien District)
- 2 In the middle portion of the Republic (Port au Prince)
- 3 In the South (Jacmel District)

These districts are in charge of the following Medical Officers of the United States Navy (1) Lieut Com R H Luning (2) Lieut W F Kennedy, (3) Lieut Com R P Parsons.

A glance at a relief map of Haiti will show that this is a geographic cross section of the Republic. The area covered in this study represents about one quarter of the Republic, that is to say about 2,500 square miles. The total area of the Republic of Haiti is 10,204 square miles. It is a little smaller than the State of Maryland. On the map the 74th meridian of west longitude represents fairly accurately the boundary between the two Governments of the Island of Haiti, the Republic of Haiti and the Republic of Santo Domingo. Recall the fact that no part of the Republic is more than a few miles from the sea and, therefore, from one of the many ancient port towns. Sixty to sixty five miles would perhaps represent the greatest distance that one could be from the sea and still remain in the Republic of Haiti. Recall also the fact that no people on earth travel more within their little realm than the Haitians, both women and men. It is nothing for a company of Haitian women of the peon class to transport by mule, or horse and upon their heads market products from the little town of Saltrou on the Eastern end of the South coast of Haiti right over the highest mountain ridge of the Republic (Morne La Selle the trail passes at a height of 6,200 feet) down off the North slope of this mountain into a desert, and then away off to the West through the Plain of the Cul de Sac to the city of Port au Prince. The distance is probably one hundred and fifty miles. The total of their market sales would perhaps be only a few gourdes. The women, however, have had "their hour" compensating for all the trouble and labor involved in the trip in meeting friends along the way and in the dicker and trade in the open market of Port au Prince. The same thing happens in every little mountain valley and gorge in the Republic the adult population is in constant daily contact with the markets in the port towns. This has been going on for at least two centu-



ness. Now let us recall the third fact which has bearing upon the epidemiology of Haitian treponematosis. Among the peon classes in the Republic sexual promiscuity is the rule. Formal wedlock is rarely entered into, but the children of looser unions are considered legitimate. With this remarkable state of affairs, is it not overwhelmingly suggestive that venereal chancreles are so rarely noted? But this is the same thing noted by physicians in Guam, Samoa and the Philippine Islands. There are two possible explanations for it. (1) That the population is so shot through with syphilitic virus that venereal syphilis can rarely find an infectable victim, and (2) that the venereal sores are overlooked. Our opinion is that the first is the correct interpretation, for not only do the skilled examiners of the Haitian Public Health Service, who are carefully examining for venereal chancreles in every part of Haiti rarely come across one either in a man or a woman but this also has been our experience in many different parts of the tropical world. This observation it seems to us should serve to settle negatively the assertion that yaws and syphilis are mutually superinoculable as far as human beings are considered.

The following is the questionnaire above referred to. We shall deal briefly with the answers received.

My dear Doctor —

We are trying to gather evidence regarding syphilis and yaws and their possible relationship. We would appreciate very much if you would give us your observations according to the following scheme:

1. Distribution (towns or rural sections)

2. Age groups affected

3. Immunity

Have you seen an individual with actual yaws or frank sequelae of yaws, such as crab, present a chancre?

4. What is your experience with congenital treponematosis? Is it common in your district?

Are you able to differentiate between congenital syphilis and yaws in a young infant?

If so, how? Do you believe that yaws may be congenital?

5. How do you differentiate between yaws and syphilis?

6. Have you by any chance seen the transition from secondary to tertiary yaws?

In answering the above questions please do not consult textbooks but give impressions you have received from your own experience.

In order to illustrate the heavy incidence of infection, the following figures may be quoted from the January report of the Public Health Service of Haiti. During this month 30,976 outpatients were seen throughout the Republic, of this number 14,997 received injections (neoarsphenamine sulpharsphenamine or bismuth) for treponematosis.

Regarding question 1. There is complete agreement that the yaws syndrome is largely confined to rural districts and small villages.

2. Most any age may show the frameside but it is chiefly seen in children.

3. All agree upon the rarity of the venereal chancre.

4. All agree that congenital syphilis occurs in their respective districts especially in the larger towns. One physician states that he never has

seen the picture of congenital syphilis as observed in the United States (Why has he not? There is admittedly plenty of syphilis in his district Why do not the mothers abort and why do not the offspring show congenital deafness, interstitial keratitis, and Hutchinson's teeth? There's the rub—native congenital syphilis does not react like European any more than the adult varieties do)

The differentiation between yaws and congenital syphilis in infants was stated to be nearly impossible where the history of extragenital primary lesion is not apparent The answers to the question of yaws being congenital were very vague

5 The differentiation between yaws and syphilis followed the lines of the ordinary textbook All agreed, however, that in the third stage the most important differentiating point was the history

6 None had seen it

The important conclusion that can be drawn from the experience of the above men is that chancres in individuals with yaws, if they ever occur, are to say the least very rare These men have seen only three cases of primary genital syphilitic sores in individuals who *apparently* gave histories of having had yaws When the thousands of cases they have had under observation are taken into consideration the rarity of such an infection is evident

In conclusion we would like to say that we have seen several examples demonstrating the truth of Hutchinson's statement that in his experience those Europeans who contracted yaws in the tropics returned home with syphilis

REFERENCES

- ¹Butler, C S Some Facts and Some Fancies Regarding the Unity of Yaws and Syphilis, U S Nav Med Bull, 1914, viii, 561-571
- ²Idem The Application of Wassermann's Reaction to the Solution of the Etiology of Tropical Ulcerations, U S Nav Med Bull, 1915, ix, 51-62
- ³Idem Discussion of J C Palmer The Relation Between Syphilis and Yaws as Observed in American Samoa, Am Jour Trop Med, 1922, ii, 349-352
- ⁴Chesney, A L, and Kemp, J E Experimental Observations on the "Cure" of Syphilis in the Rabbit with Arsphenamine, Jour Exper Med, 1924, xxxix, 553-564
- ⁵Jahnel, F, and Lange, Johannes Ein Beitrag zu den Beziehungen zwischen Framboesie und Syphilis die Framboesieimmunität von Paralytikern, München med Wchnschr, 1925, lxxii, 1452-1453 (Quoted from Stannus, 1926)
- ⁶McArthur, D C Syphilis as We See it Among the Natives of Bechuanaland Today, Am Jour Syph, 1923, vii, 569-607
- ⁷Nichols, Henry J Experimental Immunity in Syphilis and Yaws, Am Jour Trop Med, 1925, v, 429-437
- ⁸Stannus, H S Yaws and Syphilis A Critical Review, Trop Dis Bull, 1926, xiii, 1
- ⁹Vogtlin, Carl, and Dyer, Helen A Reinoculation as a Criterion of Cure of Experimental Syphilis, with Reference to Arsphenamine, Neoarsphenamine and Sulpharsphenamine, Pub Health Rep, 1925, xl, 2511-2519

HEMOGLOBIN AND ERYTHROCYTES IN THE SOUTH*

By LEON S. LIPPINCOTT, M.D., VICKSBURG, MISSISSIPPI

LAST year before this society I attempted to show that the average total and differential leucocyte counts in Mississippi differed somewhat from the textbook average normal standards. It was my belief that if studies could be carried out in various parts of the country as has been done in a few instances we would either have to accept different standards for different localities, or if these studies showed agreement the usually taught standards would have to be revised. It was my belief that we have been too ready to accept standards set down in early studies as hard and fast rules for all time to come.

A further study of a series of hemoglobin determinations and erythrocyte counts is here presented. Most textbooks give the average normal hemoglobin percentage as 100—this point varying somewhat with the method used for determination, and the average normal erythrocyte count as 5,000,000 for adult males and 4,500,000 for adult females.

The present series includes 1861 hemoglobin determinations and 1876 red counts made during the last six years on hospital and clinic patients, most of them as a part of routine laboratory examinations. The records were taken as they came without attempt to pick cases, except that hemoglobin readings below 60 per cent and red counts below 3,000,000 were not included. The findings from white and colored individuals were listed separately in order to determine any possible differences due to race.

The original hemoglobin readings were made with the Tallqvist scale because this method is less time consuming than most of the others in use. I recognize the fact that an objection may be raised to the figures obtained because of the reputed inaccuracy of the Tallqvist method. However a careful check with hemoglobin determinations made by the Newcomer colorimetric method in five hundred cases during the past year has shown Tallqvist readings, when the hemoglobin is above 50 per cent to be more accurate and consistent than is ordinarily supposed. We found in the five hundred comparative readings that the Newcomer method gave an average percentage seven points higher than the Tallqvist scale. We believe that this higher reading is probably more nearly correct and in the tables given we have added seven points to the Tallqvist readings.

The average hemoglobin determinations and erythrocyte counts in this series are as shown in Table I.

It will be noted that the hemoglobin even when the Newcomer difference of seven points is added, and the erythrocyte counts are all lower than the

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 1, 1916 and 1, 1917.

TABLE I

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER CASES	TALLQVIST AVERAGE	CORRECTION PLUS 7	NUMBER	AVERAGE
White Males	716	78.20	85.20	639	4,539,000
White Females	725	74.71	81.71	779	4,253,000
Colored Males	214	76.28	83.28	232	4,428,000
Colored Females	206	74.26	81.26	226	4,140,000
Total	1861			1876	

usual textbook standards. I have to anticipate another possible objection to the figures obtained—that these findings are from hospital and clinic patients and are, therefore, not normals. While this is partially true, few included could be actually classed as anemia, and no case of anemia as shown by complete blood picture was considered. It was also found that when the color index is computed according to the usual standards, the diminution in hemoglobin while slightly more marked, in general corresponds in degree with the diminution in red cells. This is shown in Table II.

TABLE II

	COLOR INDICES	
	WITH TALLQVIST READING	WITH NEWCOMER READING
White Males	0.87	0.95
White Females	0.79	0.86
Colored Males	0.87	0.95
Colored Females	0.81	0.88

All of the color indices computed with the Newcomer standard for hemoglobin are slightly below one.

In this series, hemoglobin of 100 per cent was found in white males in 716 determinations but three times (0.42 per cent) by the Tallqvist standard and 14 times (1.96 per cent) by the Newcomer standard. In 214 determinations in colored males, hemoglobin of 100 per cent was not found at all by the Tallqvist standard and was found but three times (1.40 per cent) by the Newcomer standard. Hemoglobin of 100 per cent was not found at all in 725 determinations in white females and was found once only (0.48 per cent) in 206 determinations in colored females, thus last being 100 per cent by both methods.

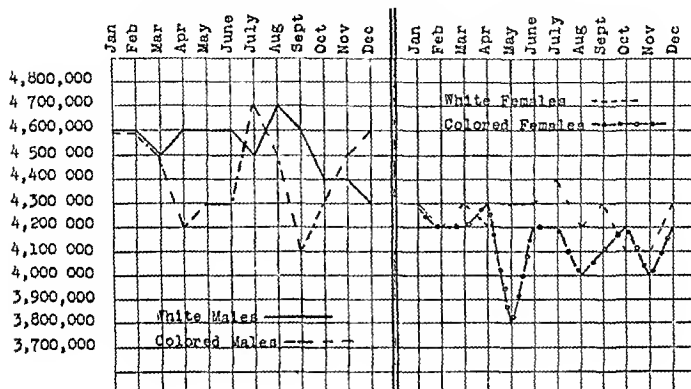
The standard 5,000,000 red cells was found in white males 117 times in 639 counts (18.31 per cent) and in colored males 32 times in 232 counts (13.79 per cent). The standard 4,500,000 red cells was found in white females 209 times in 779 counts (26.83 per cent) and in colored females 43 times in 226 counts (19.03 per cent).

Colored males show both hemoglobin and red cells slightly lower than is found in white males, and colored females show similar findings as compared with white females.

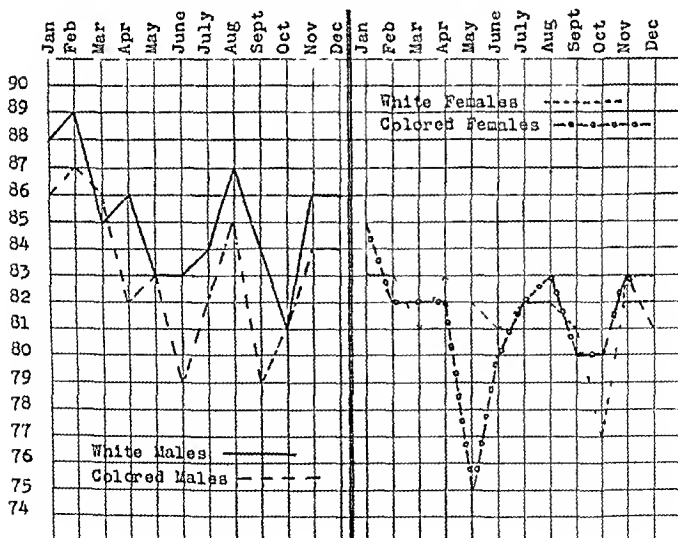
Working in the North some years ago, I do not recall that there was any reason to question the usual textbook normal standards for hemoglobin and erythrocytes. If these standards are correct for the North and the hemoglobin and erythrocyte counts are lower in the South as indicated by

the figures here given, then there must be a reason. Such a reason is not plainly evident in the literature that I have been able to find. It has occurred to me that the climate being warmer in the South less oxidation is necessary to keep up the body temperature. As hemoglobin is the oxygen

ERYTHROCYTE COUNTS BY MONTHS



HEMOGLOBIN DETERMINATIONS BY MONTHS



carrier and the erythrocytes carry the hemoglobin, less of both of these blood components might be required

In line with this reasoning the present series of determinations was listed by months to show any possible differences between hot and cold periods. The results are shown by the accompanying figures and in Table III.

In general it is shown that the hemoglobin is highest in January and February, which are our coldest months, with irregular reductions to October, followed by a rise in November and December. The colored females show a reduction in December, which is different from the others. This is probably not an accurate average finding. It is interesting to note that there is a rather sharp rise in August, one of our hottest months, in all classes.

The erythrocytes do not show the same changes as the hemoglobin, although there is considerable variation in the heights of the curves. All, with the exception of the colored females, show the highest points in July and August, and the colored females show a rise at that time.

The averages by seasons are given in Table III.

TABLE III
HEMOGLOBIN DETERMINATIONS BY SEASONS (PER CENT)

	DEC - JAN FEB	MAR - APR MAY	JUN - JUL AUG	SEPT - OCT NOV	NOV APR	MAY OCT
White Males	87.43	84.54	84.84	83.97	86.43	83.97
White Females	82.94	81.93	81.58	80.39	82.55	80.83
Colored Males	85.73	83.63	82.23	81.50	84.82	81.74
Colored Females	82.57	79.75	81.64	80.87	82.40	80.11

ERYTHROCYTE COUNTS BY SEASONS

White Males	4,506,000	4,555,000	4,600,000	4,493,000	4,508,000	4,669,000
White Females	4,272,000	4,281,000	4,305,000	4,157,000	4,241,000	4,266,000
Colored Males	4,610,000	4,341,000	4,397,000	4,263,000	4,503,000	4,352,000
Colored Females	4,277,000	4,104,000	4,134,000	4,093,000	4,194,000	4,085,000

Here we find the hemoglobin highest in December, January, and February, the coldest months, and lowest in September, October, and November, the end of the hot period. Also the hemoglobin is higher during the six months from November to April than from May to October.

TABLE IV
WHITE MALES

HEMOGLOBIN (PER CENT)				ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	94	81.06	88.06	96	4,639,000
February	76	81.64	88.64	68	4,608,000
March	38	77.50	84.50	35	4,505,000
April	60	78.83	85.83	31	4,600,000
May	39	76.28	83.28	29	4,560,000
June	45	76.33	83.33	26	4,587,000
July	64	77.42	84.42	47	4,494,000
August	68	79.85	86.85	72	4,719,000
September	57	77.46	84.46	52	4,636,000
October	58	74.48	81.48	61	4,418,000
November	53	78.96	85.96	55	4,426,000
December	64	78.59	85.59	67	4,271,000
Totals	716			639	

TABLE V
WHITE FEMALES

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	69	76.23	83.23	08	4,275,000
February	94	75.93	82.83	97	4,233,000
March	56	73.90	80.95	58	4,281,000
April	60	75.58	82.58	66	4,235,000
May	57	75.20	82.26	58	4,326,000
June	5	73.77	80.77	63	4,325,000
July	61	74.51	81.51	65	4,425,000
August	54	75.40	82.40	63	4,165,000
September	61	74.26	81.26	66	4,061,000
October	47	70.00	77.00	53	4,094,000
November	60	75.92	82.92	67	4,115,000
December	50	75.66	82.66	57	4,306,000
Total	700			719	

TABLE VI
COLORED MALES

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	31	78.87	85.87	30	4,632,000
February	27	80.00	87.00	27	4,580,000
March	27	78.80	85.80	28	4,529,000
April	15	75.00	82.00	14	4,210,000
May	5	76.00	83.00	9	4,283,000
June	19	72.37	79.37	18	4,278,000
July	13	75.00	82.00	13	4,632,000
August	6	78.33	85.33	13	4,535,000
September	16	72.31	79.31	16	4,056,000
October	16	74.39	81.39	20	4,277,000
November	27	76.80	83.80	28	4,456,000
December	17	77.13	84.13	16	4,618,000
Totals	214			232	

TABLE VII
COLORED FEMALES

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	19	71.63	78.63	21	4,269,000
February	18	73.27	80.27	19	4,206,000
March	11	74.55	81.55	11	4,197,000
April	14	75.30	82.30	12	4,267,000
May	6	68.33	75.33	9	3,848,000
June	15	73.33	80.33	14	4,227,000
July	26	75.00	82.00	27	4,168,000
August	1	76.19	83.19	22	4,009,000
September	18	72.50	79.50	21	4,078,000
October	15	73.33	80.33	20	4,182,000
November	20	75.77	82.77	31	4,019,000
December	17	73.82	80.82	19	4,207,000
Totals	206			226	

The erythrocyte counts are highest in the period from June to August and lowest in the period from September to November in whites, both male and female, and highest in the period from December to February and lowest in the period from September to November in negroes, both male and female. Also in the six month periods the red cells are higher from May to October in whites and from November to April in colored.

It is further interesting to note that of the hemoglobin determinations showing 100 per cent, 12 of the 14 in white males occurred in January and February, all three in colored males occurred in January and February, and the one in colored females occurred in December. The normal standard red counts found occurred more frequently in the colder seasons with the exception of those found in colored females, in whom season does not make much difference.

CONCLUSIONS

These studies indicate

1 The normal average hemoglobin percentage and erythrocyte counts are lower in this locality (Mississippi) than the usually accepted normal standards

2 The normal average hemoglobin percentage is approximately 84 per cent for males and 81 per cent for females, when the Newcomer method of determination is used

3 The normal average erythrocyte count is approximately 4,500,000 for males and 4,200,000 for females

4 Color indices average slightly below one

5 In general, hemoglobin is highest in cold months of the year and lowest at the end of the hot period

6 While erythrocyte counts do not change with the seasons as does the hemoglobin, most of the higher counts occur in the colder months

7 There is a real need for a hemoglobin standard and a standard method of determination

8 There is also a real need for the determination of what constitutes a normal erythrocyte count

REFERENCES

- Chamberlain, Weston P. Observations on the Influence of the Philippine Climate on White Men of the Blonde and of the Brunette Type, *Philippine Jour Sci*, December, 1911, vi, Sect B, p 440
- Culpepper, W L. Blood Changes in the Aviator, *Mil Surgeon*, 1921, xlviii, 180
- Greene, Charles Lyman. Practice of Medicine, Frederick Tree, M D, Hagerstown, Md, W F Prior Co, Inc, 1925, vi, Sect ix, Chap 1, p 742
- Haden, R L. The Normal Hemoglobin Standard, *Jour Am Med Assn*, Oct 28, 1922, lxxix, 1496
- Idem. The Need for a Fixed Hemoglobin Standard, *JOUR LAB AND CLIN MED*, April, 1926, xi, 696
- Macleod, J J R. Physiology and Chemistry in Modern Medicine, St Louis, 1922, C V Mosby Co, Chap XI, pp 92-93
- Mallory, Frank B. Principles of Pathologic Histology, Philadelphia, 1914, W B Saunders & Co, p 21
- Norgaard, A, and Gram, H C. The Normal Relation Between Hemoglobin and Corpuscles, *Ext, Jour Am Med Assn*, Feb 10, 1923, lxxx, 444
- Phalen, James M. Tropical Climate and Its Physiological Effects, *New York State Jour Med*, Nov, 1912, xii, 627
- Stitt, E R. Practical Bacteriology, Blood Work and Animal Parasitology, Philadelphia, 1918, P Blakiston's Sons & Co, pp 225, 227, 252, 253
- Wright, James Homer. Nelson Loose Leaf Medicine, New York, Thomas Nelson & Sons, iv, Chap 1, p 3

DISCUSSION

Dr C L Spahr—During the discussion of a paper by Drs Lindsay and Rice, and read at last year's meeting, points were brought out, favoring the use of reliable hemoglobinometers and the recording of the findings by grams per 100 cc of blood. I regret that the

author of today's paper has seen fit to make his hemoglobin determination by the Tallqvist method and to record them in per cent

Dr Herman Spitz—I would like for Dr Lippincott to tell us what investigations he has carried on in regard to the question of intestinal parasites in these cases especially hookworm infestation Also as regards chronic malaria It would be interesting to know the incidence of these diseases during childhood and adolescence and what connection there is between these diseases and their accompanying malnutrition and the low values obtained by Dr Lippincott

Dr E F Cooke—I have not looked over my counts carefully since the program was received I have rather the impression that there is a very marked degree of truth in the main in Dr Lippincott's paper Whether it is from being much further south than Nashville, Tennessee or not I do not know With the Tallqvist scale we run approximately 80 per cent for an average I ordinarily use a Dure hemoglobinometer with which I am not satisfied, I am looking for something better With the Tallqvist it has been my custom to add 10 per cent I think that we do have a moderate decrease in the erythrocytes and hemoglobin in the South I think I have noticed that it is Sometimes people who have recently been in the mountains have a higher count and a higher hemoglobin than our people that have stayed home during the hot season I do not think the difference between the negroes and the whites is of much importance I do think that on the main Dr Lippincott's contention is correct, that there is a diminution of erythrocytes and hemoglobin as you go South

Dr F E Soderen—I am quite in sympathy with Dr Lippincott's views Of course I speak for a different part of the country and for a different class of people but the 100 per cent hemoglobin and the five million erythrocytes are distinctly the exception and not the rule Our average figures are considerably below these as might be expected in the class of city dwellers with which we have to deal

Dr Lippincott (closing)—I am very much interested in the whole question of blood counts I agree with Dr Spahr and accept his criticism the hemoglobin should be given in grams per one hundred c c I did not do this because the majority of people understand the 100 per cent standard better In regard to what Dr Spitz said we did not go into the history of each of these cases they were merely routine laboratory cases Last year there was an extremely low percentage of intestinal parasites I am now looking over some figures on malaria and here again State Board statistics indicate too high an incidence The iron is an important point I had not thought about the metabolism that may explain much of the difference noted I want to thank you for your discussion

LABORATORY METHODS

DROP-RECORDERS*

By O S GIBBS, HALIFAX, N S

SINCE every physiologist and pharmacologist is, will be, or has been, faced with the problem of recording the outflow or inflow of fluid, perhaps the following short review of the various types of available drop recorders may be of some service. No claim is made, however, that this article is complete, especially as in most cases the origin of an instrument is obscure, and many modifications of any one type exist.

At least five principles are used in drop-recorders, namely

Impulse
Weight
Expansion
Electrolytic
Displacement

apart from somewhat impractical methods such as photographic, or changes in electrical capacity.

The first type consists of some kind of a platform,¹ either balanced or held by a light spring, onto which the drop falls. Energy is thus imparted to the platform which is depressed, and thus either makes or breaks a circuit. This form of instrument is simple, which is the best that can be said for it. On the other hand it requires very careful adjustment, with a sufficient distance for the drop to fall, which is often very inconvenient, and it will not work satisfactorily if the viscosity of the fluid changes appreciably.

One instrument which had, however, an arm of about 20 cm length responded fairly well, providing the circuit was broken by the falling drop.

Weight of the drop is utilized in several forms of instruments. The best of these has been devised by Condon.² In this instrument the drop is caught on a short counter-balanced platinum spiral, as it runs down this it momentarily adds its weight, thus causing it to tilt and close a contact. This instrument is simple and small, it works very well if properly adjusted. Unfortunately it fails if the fluid to be measured changes its viscosity to any extent. Without doubt it is a useful device for such work as urine flow in cats or rabbits. This instrument is often confused with the impulse variety, from which it is entirely distinct, since it works just as well from a bare clearance of the dropping tube, as from several centimeters height.

*From the Department of Pharmacology Dalhousie University Halifax N S
Received for publication November 26 1926

Another crude and very simple form also devised by Condon (unpublished) is as illustrated in Fig. 1.

It is a tube held firmly in a clamp and connected by a piece of fine rubber tubing *B* and *C* which is a piece of thin glass tubing. As a drop forms on the end of *C* the extra weight bends down the tube which springs back to its original position when the drop falls off. In this way each drop is recorded. A well made instrument of this type will function with all kinds of fluids, if somewhat imperfectly.

The third or expansion type has not been experimented with to any great extent. An example is illustrated in Fig. 2. *A* is a small half cone bent out of thin metal. This is pivoted on a light arm the counter end of which rests against a light spring contact *X*. The face of the cone rests against a piece

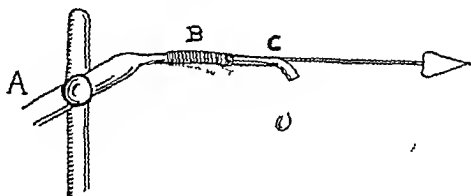


Fig. 1—Natural size

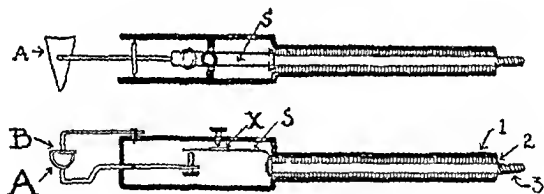


Fig. 2—Natural size

of flat metal *B* which is fastened to the frame of the instrument. As a drop falls in the cone it first fills up (2 to 3 drops) the next drop in order to escape, forces the cone away from the plate and thus closes the contact *X*. Owing to surface tension effects a spring is not required in order to pull the cone back to the plate. This instrument has one advantage over most of the mechanical type for the moving parts are as it were on edge in consequence of this they can be made much heavier than is usual since very little power is required to move them. This renders the instrument less liable to accidental damage beyond this there is little to choose between it and the Condon type since they both fail if the fluid changes its viscosity.

Expansion of a filling tube (ven) has been taken advantage of by Gesell³ in his ingenious device which no doubt could be made to function as an efficient drop recorder. It is however somewhat complicated and also very expensive.

The fourth principle is that of causing the drop to fall between two electrodes, thus itself closing a circuit. Obviously this can only be successful if the fluid conducts electricity sufficiently well. Unfortunately for practical purposes urine alone, and even that not constantly, will work directly with this principle. It can be used with great success for measuring inflow, since most fluids injected intravenously are made up with Ringer or some such solution. For this, and any other purpose where the fluid is suitable, the apparatus shown in Fig 3 works very well.

The principle used is that of the ordinary "sight feed" lubricator, with the addition of the necessary electrodes *A* and *B*. *A* passes from the upper terminal to the dropping tube, and projects about 1 cm. downwards. *B* enters the chamber at right angles, but is bent down parallel to *A* at just sufficient distance from it that the down-falling drop touches both, but is not checked. In this way the circuit is momentarily completed. This instrument works

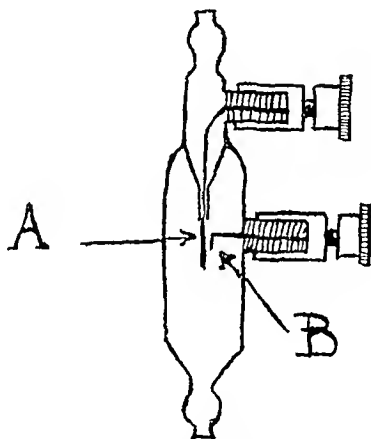


Fig 3—Simple form of recorder

quite well up to 1000 drops per minute (about 30 cc), providing a good relay is inserted in the circuit, see tracing (Fig 7).

The above apparatus is readily constructed from "Pyrex" tubing, but as will be easily appreciated it is almost impossible to make two dropping tubes exactly the same. As it is very useful to have two instruments working together it was decided to construct one in which the size of the drop could be altered. Two possibilities offered themselves, either increasing the dropping surface, or by elongating the dropping surface, thus causing the drop to break quicker. This latter procedure was adopted as appearing easier to make, and proved itself quite satisfactory. The final apparatus is as shown in Fig 3-A, in which a fine threaded rod passes through the cap *M* to the dropping point, thus by simply screwing the rod down and increasing the length of the dropping surface, the drop breaks off sooner, and vice versa. A little extra complication is introduced since the electrode *B* must also be capable of vertical movement in order to be adjusted for the new dropping position. This was not difficult to accomplish as will be seen from the sketch Fig 3-A.

Such an instrument as described above has many obvious advantages over the preceding ones, there being no mechanical parts to get out of order. Clearly its use is limited by the fact that many body fluids do not conduct electricity sufficiently well to work it successfully even if a high voltage be used and that in itself is apt to be disadvantageous. Also if the fluid changes its rate of viscosity the drops will vary in size and thus impair the accuracy somewhat.

These facts have led to the construction of a very beautiful instrument by Hanke in which at least one very important advance was made. This in

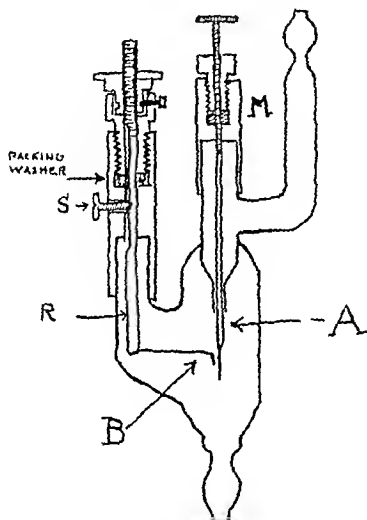


Fig. 4.—Form with drop adjustment. The terminals are not shown in sketch they are soldered onto the side of the brass caps. A teeth flat on the rod *F* onto which set screw *S* bears. This is to prevent electrode *B* twisting.

strument utilizes the electrolytic principle but in place of the body fluids acting directly they are used to displace 10 per cent sodium sulphate solution from another container. Secondly the receiving electrode is connected to a slight minus pressure, thus ensuring a regular size of drop, and a clean contact break. Properly constructed this instrument has a very remarkable accuracy (it is claimed to $\frac{1}{2000}$ th of a cc) and unquestionably fulfils most of the requirements of a good recorder. Since it works primarily by displacement the viscosity of the fluid to be measured is of no importance which is of moment in salivary work for which the instrument was first devised. Its size is not objectionable since fluid may be conveyed to the apparatus by a tube, which, especially if filled with fluid does not impair its accuracy.

As this type has not been described in detail other than in Russian, apart from a cursory mention by Anrep⁴ I venture to give the details of one form that works satisfactorily. Although this instrument is unquestionably somewhat complicated it is compensated for by ease of working. Fig 4 is a photograph of the apparatus. *A* is the receiving vessel for the fluid to be measured, which for convenience has a drainage cock at the bottom as well as a shut off cock at the top. The vessel is connected by means of a three-way tap to the sulphate vessel *B*. When in working position the fluid displaces air from *A* and forces sulphate up the tube *C* to the electrode *D*. This electrode is a concave platinum plate about 5 mm in diameter with a small hole in the center. The drop forming on *D* eventually touches the lower or suction electrode *E* and at that moment makes contact, thus activating a relay. *E* is a slightly larger concave plate with a somewhat larger hole in the center. It is connected

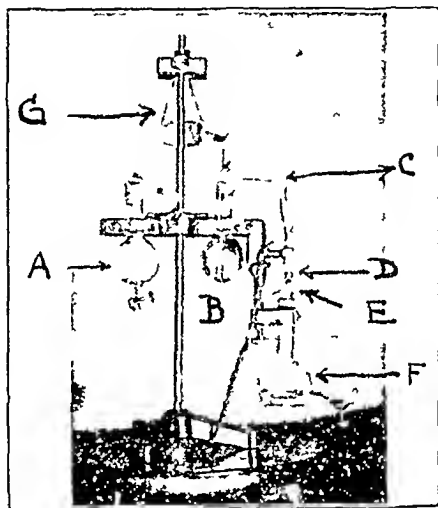


Fig 1—Complete apparatus with suction electrode for outflow records 100 c.c. capacity

through the flask *F* with a filter pump. Flask *G* is merely a reservoir of sulphate, which is used to fill the vessel *B* by means of the two three way taps. *A* also can be emptied without disconnecting the animal, by means of its drainage cock and the three-way tap *T*. The sulphate solution can of course be used repeatedly. Where sudden changes of flow are expected it is advisable to fill the air-space with petroleum ether, or some such light insoluble fluid, since in an instrument of 100 c.c. capacity equilibrium takes a moment or so to establish.

Hanke's suction electrode is not applicable for measuring inflow, and the above type can only be used for recording outflow. Its displacement principle can be used in conjunction with the electrolytic type previously described even nonconducting, or viscous fluids can be accurately recorded. For this purpose, and for most others, the following type of instrument will satisfy all the usual requirements. (Fig 5)

A is the displacement chamber connected to a reservoir *B* by means of a

three way cock which also serves as an outlet. Fluid is displaced from this chamber by means of the expansion of a fine rubber condom *C*. If the instrument is to be used as an inflow recorder (Fig 5) sulphate solution (from a constant level device) is run into the condom passing on its way through an electrolytic recorder of the type already described (Fig 3). The expansion of the condom naturally forces out fluid from the chamber in exactly equal amount to that entering the condom which will contain over 120 cc without stretching. For outflow records, on the other hand, the chamber is filled with sulphate solution, which on being displaced by the expanding condom which

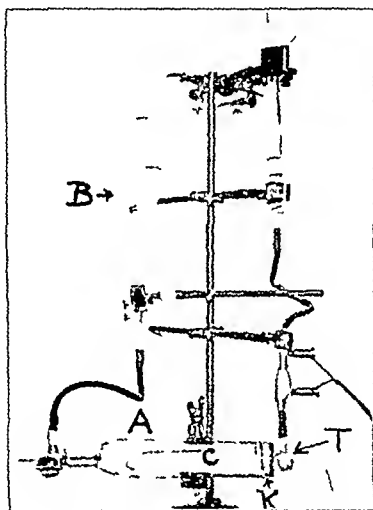


Fig 5—Arrangement for measuring inflow

is connected to the animal runs through a recorder now connected with the tap *D* (Fig 6)

The above form of displacement chamber is almost ideal since relatively large quantities of fluid may be displaced with but little change in hydrostatic pressure. Furthermore it readily adapts itself for use with warm fluids. Since excretion may be required for analysis a special method is adopted for fastening the condom in order to facilitate its removal. This consists of turning down the end of the rubber cork *K* so that it is quite free from the walls of the chamber. On this a fairly deep groove is cut. The condom is pulled over this groove and fastened in position by a rubber band. In this way not only may the condom and its contents be readily removed but by leaving a clearance between it and the chamber walls it is not apt to become torn in pushing the cork well home. The practice of jamming the condom between

REFERENCES

- ¹Macmillan, B R Quart Jour Exp Physiol, 1913, vi, 109
²Condon, N E Proc Physiol Soc, Jour of Physiol, Dec 17, 1921
³Gesell, R Am Jour Physiol, 1924, lx, 254
⁴Anrep Jour Physiol, 1920, lxx, 372

DESCRIPTION OF A NEW HYDROGEN-ION COLORIMETER

BY R B H GRADWOHL, * M D, St Louis, Mo

IT IS a matter of prime necessity for bacteriologists to adjust accurately the reaction of their culture media. Formerly, accurate quantitative measurements of hydrogen ions by the colorimetric method were dependent upon variable factors which many times led to erroneous readings. For example, buffer and indicator solutions were subject to deterioration, due to the growth of organisms. Indicators varied as to purity, and the intensity of their color changed markedly even in buffered solutions hermetically sealed. Research has gradually solved these troublesome problems, and with the advent of standardization of dyes, manufacturers have today placed on the market highly refined indicators. With materials and procedures standardized, it is now possible to offer accurate colorimeters for the measurement of hydrogen ions.

The electrometric method of determining the concentration of hydrogen ions is the ultimate standard of comparison, but since such a method is costly and requires a trained worker to follow accurately the various steps of the process, the colorimetric method holds sway. If buffered solutions are accurately checked by the electrometric method, growth of organisms prevented in the same, and the indicators of the highest purity used, then it is possible mechanically to approach closely by colorimetry the accuracy of the electrometric method.

The time honored method of determining the degree of acidity and alkalinity by titration has been discarded in most instances, due to its fallacies. Since the degree of acidity or alkalinity depends on the concentration in solution of H^+ ions, it necessarily follows that a satisfactory method of determining the degree of acidity or alkalinity must accurately measure quantitatively the H^+ ions in solution. This quantitative measurement is accomplished by determining the voltage produced in a solution containing hydrogen ions—each H^+ ion carrying an unvarying amount of electric energy. By means of a calibrated electric equipment we are able to measure accurately the amount of energy produced by H^+ ions in solution and subsequently to determine the number of these necessary to produce such energy.

In the colorimetric method of measuring hydrogen ions, buffer solutions play an important part. In general a buffer solution is one which is able to assimilate definite amounts of free acid or alkali without changing the con-

*Director Gradwohl Laboratories

centration of the H^+ ions in solution. Since solutions absorb CO_2 from the air, which tends to make them acid, and also absorb all oils from glassware, it is important that once a solution is checked electrometrically it remains accurate.

Clark and Lubs buffers meet the requirements. In certain of the buffers, however, molds are able to utilize the inorganic salts as food, and as a consequence the buffer after a certain period gradually becomes inaccurate. Hence it is of prime importance to render these buffers sterile and be assured they will stay so. This is a problem that has but recently been solved.

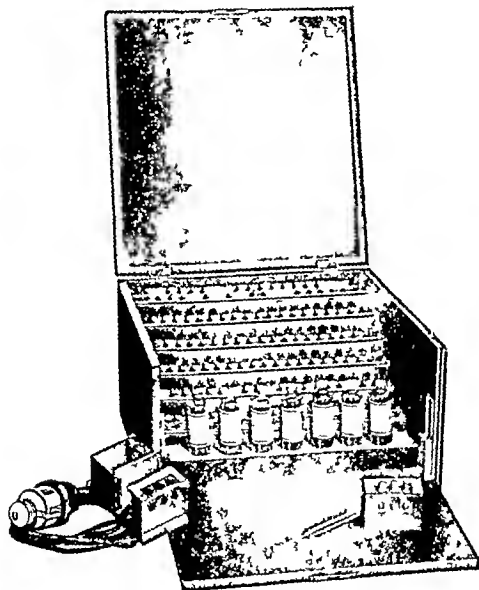


Fig. 1—The Gradwohl colorimeter

Now let us discuss the buffer solutions. If all solutions were purely and entirely composed of known acids and alkalis it would be comparatively simple to ascertain readily their P_H values. Such, however, is not the case. Most solutions which we desire to compute for acidity or alkalinity contain impurities and other substances beside their integral acid or alkali values. These impurities have a "buffer action," as noted by Clark, which is "the resistance exhibited by a solution to change in P_H through the addition or loss of acid or alkali." In general the salt of any weak acid or weak base is a buffer salt. In making up an equipment to determine the P_H of unknown solutions, we must work out a series of buffers which with the

tion of the proper indicator, give us a basis for comparison with unknown buffers, plus our known indicators

Regarding indicators, it seems academic to note that they change in color when they are acted upon by solutions of different acidities or alkalities. Clark and Lubs are responsible for the development of a number of indicators which have a wide range, that is, from extreme acidity on one hand to extreme alkalinity on the other. We have used the following indicators in our work in connection with this new equipment

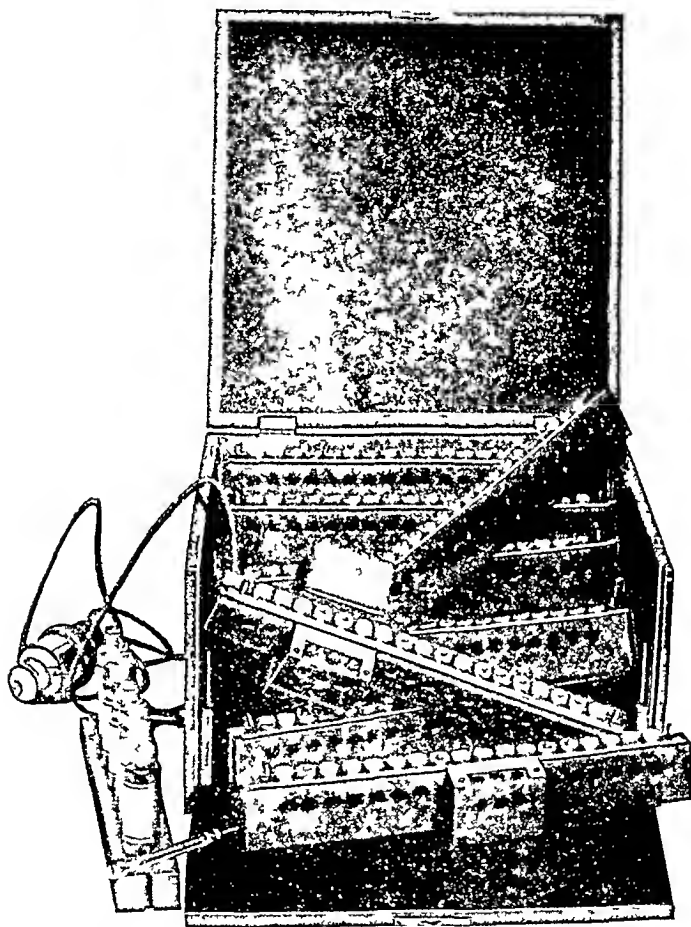


Fig 2.—Equipment for test

Thymol blue	Ranging from 12 to 28	changing from red to yellow
Bromphenol blue	" " 30 to 46	" " yellow to blue
Bromcresol green	" " 40 to 56	" " " red
Chlorphenol red	" " 52 to 68	" " " blue
Phenolphthalein blue	" " 60 to 76	" " " red
	" " 68 to 84	" " " red
	" " 72 to 88	" " " blue
	" " 80 to 96	" " " "

ed that this indicator covers two hydrogen-ion ranges one of extreme and the other of extreme alkalinity. PH 80 to 96. The color change from red at PH 12 to orange yellow at PH 28. The color change of from a greenish yellow at 80 to blue at PH 96

The equipment which we are about to describe consists of a box (Fig 1) with a series of hermetically sealed special glass ampoules containing buffers of known P_H value. The P_H value of each ampoule will be found on the metal strip running along the top of the rows. In addition to this the indicator for each row is designated by initials on the end of the rack. Next, there is a series of indicator bottles properly labelled (Figs 2 and 3). The illuminating box is furnished with an arrangement for sliding back and forth along any one of these rows of buffers which are taken from the box for comparative purposes after a rough test has been made and the actual indicator selected.

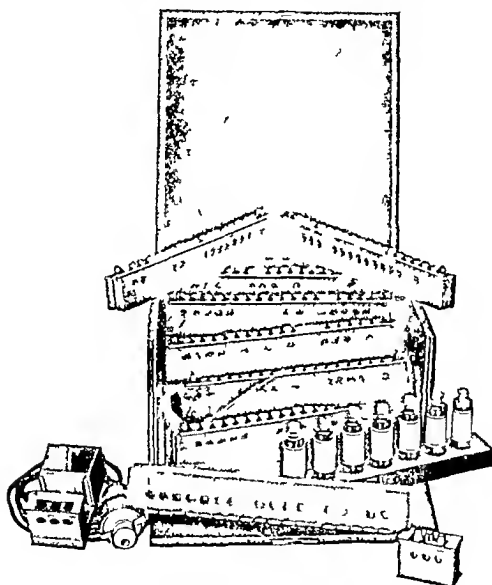


Fig 3—Another view of equipment.

Before proceeding with the rough test certain facts are to be borne in mind regarding P_H values. We know that the P_H value or rating of actual neutrality is 7.0. Any value above 7.0 such as 7.2 or 7.4 etc. denotes alkalinity. Any value below 7.0, such as 6.8 or 6.4 denotes acidity. We can change the P_H it will by adding acid or alkali as the case may be to bring the value up or down. If you have a solution with a P_H value of 6.2 it is acid. You may want to make it 7.8. You do this by adding sufficient alkali. With these facts in mind we now proceed to carry out what is known as a 'rough test' which is the effort to ascertain just what the hydrogen ion of the unknown actually is.

In a test tube place 5 c.c. of the unknown solution (Fig 4) and add four or five drops of one of the indicators, preferably using the bromthymol blue first, for the reason that this has a P_H of 6.0 to 7.6 and therefore covers the neutral point P_H 7.0 as already described. In this way we can determine at once whether the solution is neutral, acid or alkaline. We know that bromthymol blue changes from yellow at 6.0 to deep blue at 7.6. If we add brom



Fig 4—Manner of beginning rough test

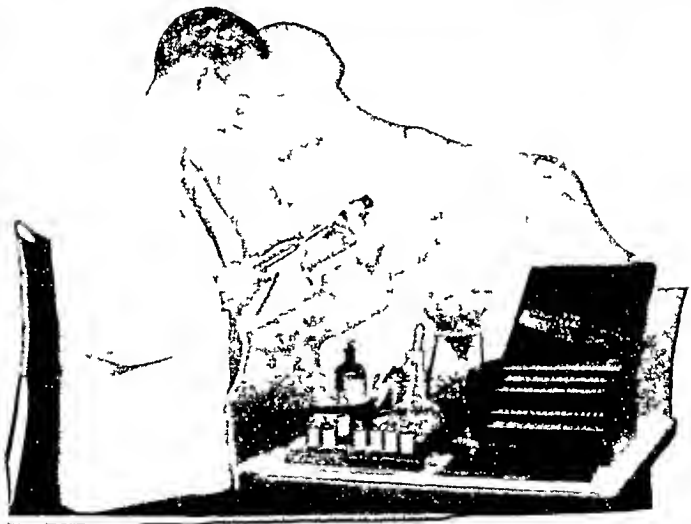


Fig 5—Method of using syringe and adaptor for withdrawal of indicator solution in exact quantity

thymol blue and we obtain a color which is between yellow and deep blue, we know at once that the P_H value of this solution lies between 6.0 and 7.6 and that it is either neutral or very slightly acid or alkaline. In other words, it is on the border line of neutrality. If a yellow color is obtained by the addition of bromthymol blue we know at once that the P_H value is 6.0 or lower. Since the P_H value must be 6.0 or lower, we next proceed to use the indicator which covers the acid range of P_H 4.0 to 5.6, which is bromeresol

green. This must be added to the second test tube containing 5 cc of the unknown solution. Color change for this indicator is from yellow at 4.0 to deep blue at 5.6. Any color between yellow and deep blue means a P_H between 4.0 and 5.6. Proceed therefore to draw your conclusions as with the previous indicator. If on adding bromthymol blue indicator solution in the first test we obtained the deep blue color the solution would be alkaline with a P_H value of 7.6 or even higher. To determine the higher value use thymol blue which ranges from P_H 8.0 to 9.6. Having in this way obtained a rough idea of the approximate range of P_H value of the unknown we proceed to make the actual test.

If you have found the range is between say 5.2 and 6.8 you place the standard amount of the unknown in a small test tube. Into this test tube

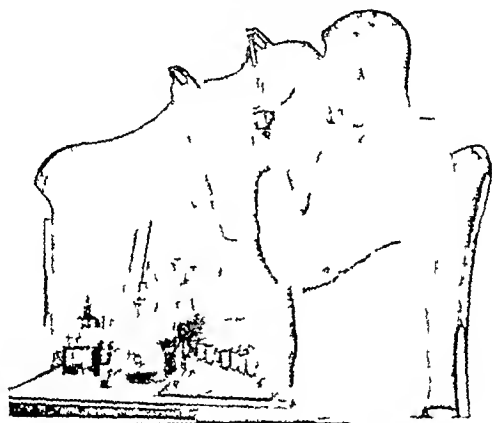


Fig 6—Adding Indicator to unknown

place 0.2 cc of indicator used in the rough test which gave you this range. This amount is withdrawn from the indicator bottle by means of the adaptor (Figs 5 and 6) which the operator attaches to his own particular glass syringe (these adaptors may be obtained from Beeton Dickinson & Co. Rutherford N. J.). Shake and place in the illuminating box. The other tubes containing the unknown solution without indicator are arranged on either side of the first tube containing the indicator and the reading made against the unknown buffers of the identical indicator (Fig 7). These buffers are taken from the box in the rack and identified by the initials on the end of the rack. When the color matches the P_H value can be read from the known buffer solution.

For the benefit of those who have not used the hydrogen ion method in adjusting the reaction of culture media we will give the following simple adjustment:

To adjust the reaction of culture media—Let us assume that we are going to adjust the reaction of the culture medium to P_H 7.6. Choose as the indicators to be used phenol red, covering the range 6.6 to 8.2, and cresol red, covering the range of 7.2 to 8.8. Place 10 c.c. of the medium in a casserole. Add 10 drops of phenol red indicator. The color will become yellow if the medium is acid, red if the medium is alkaline, orange if neutral. If acid, add to the medium in the casserole $N/10$ NaOH drop by drop from a burette until the color changes from the yellow of the acid reaction to the red of P_H 7.6, as seen in the colorimeter. Read from the burette the number of c.c. $N/10$ NaOH used to adjust the reaction of 10 c.c. of medium. Measure the total quantity of culture medium made. Say you have 1000 c.c. of culture

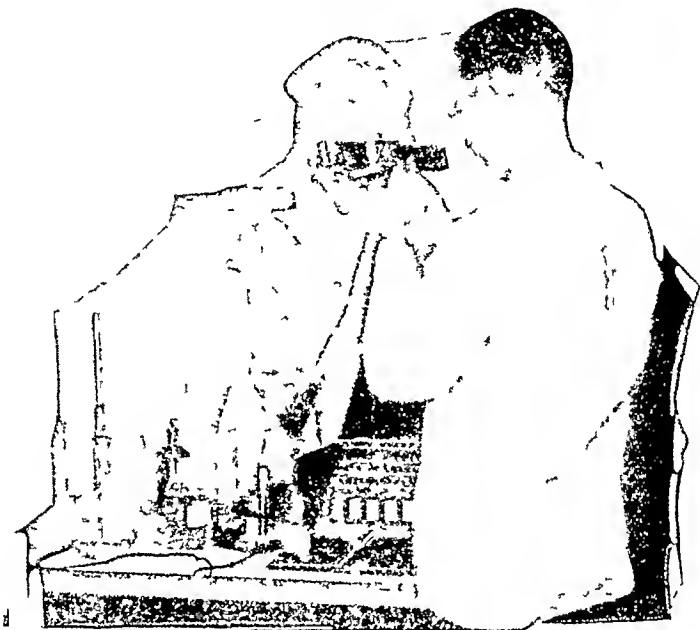


Fig 7—Making color comparison.

medium on hand, it requires 100 times as much NaOH to neutralize 1000 c.c. of culture medium as it requires for 10 c.c. Multiply the number of c.c. of $N/10$ NaOH used by 100. One-tenth this quantity is the amount of $N/1$ NaOH to be used. The $N/1$ solution is used for final adjusting so that the total volume is not changed to any very great extent. Add to the culture medium the quantity of NaOH required and mix thoroughly.

Place 5 c.c. of the adjusted medium in each of two test tubes. To the first tube add 0.2 c.c. of phenol red indicator, to the second 0.2 c.c. of cresol red indicator, mix, and compare in the colorimeter. The colors should match at 7.6 with both indicators. If the culture medium is still too acid, figure again the quantity of NaOH required to bring the reaction to the proper hydrogen ion concentration. If it is too alkaline the reaction can be adjusted in the same way by using HCl instead of NaOH.

The particular advantages of this outfit described are as follows

- 1 A series of standard buffer solutions prepared in a sterile manner which will resist deterioration
- 2 Adequate buffers covering the entire range of acidity and alkalinity suitable for any kind of industrial or bacteriologic investigation
- 3 The use of small test tubes with a minimum amount of indicator, thereby conserving the expense of purchasing fresh indicator solutions
- 4 A uniform illumination which gives a standard and equal result at all times
- 5 The mechanical arrangement of the riel's with the illuminating box which permits one to adjust it quickly to rack after rack and perform the test in the minimum amount of time

A RAPID METHOD FOR THE DETERMINATION OF CHLORIDES IN BLOOD OR URINE*

BY S. L. LEIBOFF, NEW YORK CITY

A NUMBER of methods are described in the literature for the determination of chlorides in body fluids and tissues. All these methods, with but few exceptions, are based upon the same principle of precipitating the chlorine in the form of the insoluble silver chloride by means of an excess of silver nitrate in an acid solution. The excess of silver nitrate then being determined by volumetric means with a thiocyanate salt in the presence of a ferric salt which is used as an indicator.

Van Slyke and Donlevay,² and Austin and Van Slyke¹ determine the chlorides in blood by removing the proteins with picric acid and determining the chlorine in the filtrate by titrating the excess silver nitrate with a standard potassium iodide solution using starch as an indicator.

Meyers and Short⁴ also use the picric acid filtrate of blood, they combine the three ingredients, silver nitrate, nitric acid, and ferric alum, in one solution, and after removing the silver chloride by centrifugation, they determine the excess silver nitrate in the supernatant fluid with ammonium thiocyanate.

Whitehorn,⁵ however, objects to the addition of nitric acid directly to the silver nitrate, as by doing so it may result in the mechanical enclosure of silver nitrate solution within the curds formed during the precipitation of the silver chloride, thus giving higher results. He uses the Folin-Wu⁶ tungstic acid blood-filtrate in the Volhard-Harvey³ method.

Later, Van Slyke⁷ recommended a method whereby the blood proteins are destroyed by boiling with silver nitrate in a solution of concentrated nitric acid, and the excess of silver nitrate is titrated with standard thiocyanate salt and ferric alum.

The same objection may be raised to this method as to the one of Meyers and Short, with the additional disadvantage that an excess of nitric acid might exert a slight solvent action upon the silver chloride. The method is rather lengthy as it requires from one to two hours to digest serum and even longer periods to digest whole blood.

Isaac⁸ described a colorimetric method for chlorides. He centrifuges the tungstic acid blood-filtrate of Folin-Wu⁶ with magnesium carbonate and silver chromate and compares the color in the supernatant fluid against a known standard of the same color.

Dupray⁹ improved this method by adding potassium iodide and sulphuric acid to the colored solution thus obtaining a deeper color which is more suitable for colorimetric reading.

*From the Biochemical Department of Lebanon Hospital Laboratory, New York N. Y.
Received for publication December 5, 1926.

Of the three main subdivisions of quantitative chemistry, viz, gravimetric, volumetric, and colorimetric, the gravimetric methods are usually the most accurate, but their technique is very lengthy and cumbersome, requiring great care and skill in their manipulation, so that their use in the analysis of clinical material is impractical, and in many cases impossible on account of the exceedingly small amounts of material obtainable. The volumetric procedures, while not attaining as high a degree of accuracy in all cases as the gravimetric procedures, are sufficiently accurate provided great care is taken in the preparation and standardization of the solutions. They have the advantage over gravimetric procedures in that they are very rapid.

It is quite obvious that colorimetric procedures are the least accurate, for they are dependent upon a number of varying factors, such as the stability of the color, the proper adjustment of the colorimeter, the proper source of light, and most of all, upon the personal equation of the observer. For hardly any two individuals will obtain exactly the same reading on the same solution under the same conditions nor will the same person duplicate his own reading each time.

In spite of the limitations placed upon colorimetric procedures, however, they find a great use in biologic chemistry because they furnish a method for the determination of very small amounts of substances which could not be determined by any other means. In the case of chloride determination, though, there is no advantage in using a colorimetric method, since chlorides are present in large amounts in blood and in urine thus being very adaptable to volumetric determination.

Of the various volumetric procedures here described, the Volhard-Harvey¹ method seems the most adaptable for clinical work since it is the most rapid method and gives results equal to those obtained by other methods. It, however, has the disadvantage in that the end point produced by the excess of silver nitrate with the ferric alum is not clearly defined. Also the cyanate solutions do not keep very well so that Van Slyke recommends their restandardization at least once in two weeks.²

In the method which follows the cyanate is eliminated altogether, and the chlorine is determined directly with silver nitrate.

The principle of the method is as follows:

The solution containing chlorides is made neutral with calcium carbonate and titrated with a standard silver nitrate solution in the presence of sodium chromate which serves as an indicator producing a red color when the end point is reached.

Reagents Required

- 1 Sodium chromate a 0.25 per cent aqueous solution
- 2 Calcium carbonate a fine powder
- 3 Standard sodium chloride, 0.1 per cent solution
- 4 Standard silver nitrate solution, containing 1.452 gm. of silver nitrate in a liter of water

*Preparation of Pure Sodium Chloride*¹⁰

Since in this method the silver nitrate is standardized against sodium chloride, it is necessary that the latter salt should be of the highest purity. It may be prepared by the following simple technique:

Dissolve about 500 gm of a good grade table salt in a liter of water and filter. Add concentrated HCl slowly until the chloride just begins to precipitate, and pass into the solution HCl gas until no more salt is being precipitated. When no more salt separates, filter through a Buchner filter and drain by suction. Wash with about 200 cc of concentrated HCl solution in successive small portions, allowing to drain completely after each addition of the acid. Finally wash the salt with about 50 cc of water and test this wash-water for sulphates with BaCl_2 . If sulphates are present continue the washing with HCl. Transfer the salt to a porcelain dish and heat until decrepitation ceases, cool in a desiccator for twenty-four hours and place in a well-stoppered bottle.

To prepare a 0.1 per cent NaCl solution dry a few grams of the salt in a desiccator to a constant weight, and dissolve one gram in a liter of water. This solution will contain one milligram NaCl in one cc of the solution.

PREPARATION OF STANDARD SILVER NITRATE SOLUTION

Weigh out about two grams of silver nitrate crystals and dissolve in a liter of water. This solution is more concentrated than is required. Dilute this solution so that two cc of silver nitrate solution will precipitate exactly 1 mg of sodium chloride, as follows: Into a small beaker place 10 cc of the standard NaCl solution, add about 0.3 gm CaCO_3 powder and 0.5 cc of the sodium chromate solution. Stir with a stirring rod and run in from a burette the silver nitrate solution until the first red color is obtained. The exact end-point can easily be recognized by preparing a control beaker containing 10 cc of water, 0.3 gm CaCO_3 , 0.5 cc of the chromate solution, and one drop of the silver nitrate solution delivered from the same burette, record the volume of the drop. The volume of the drop is to be subtracted from the volume of silver nitrate used to titrate the sodium chloride. To make up a liter of standard AgNO_3 solution use the following simple formula:

$$50 \times X = Y$$

- X Volume of AgNO_3 used to precipitate 10 cc of the NaCl
 Y Volume of AgNO_3 necessary to make up one liter of such strength that 2 cc will be equivalent to one cc of a 0.1 per cent NaCl solution

For example, if it took 17.5 cc of silver nitrate to precipitate the 10 cc of the sodium chloride, then $50 \times 17.5 = 875$. Thus if 875 cc of the silver nitrate is diluted with water to make up one liter, a solution of the proper strength is obtained. The standard solution should be kept in a dark bottle.

Procedure for Chlorides in Blood

Place 5 cc of the tungstic acid filtrate of Folin-Wu⁶ (0.5 gm of blood) in a small beaker. In a similar beaker place 5 cc of water. This beaker is to be used as the control for the end-point. Add to each beaker about 0.3 gm powdered CaCO_3 and 0.5 cc of the sodium chromate indicator. Add one

drop of the standard silver nitrate solution from a burette to the control and record the amount. Now run in the silver nitrate standard into the blood filtrate until a change in color is produced similar to the one in the control. Subtract from the last reading the recorded reading of the control. This is the amount of silver nitrate used up to precipitate all the chlorine from the filtrate.

Calculation of Results

$$\lambda \times 100 = \text{mg. NaCl per 100 cc. of blood}$$

$$\lambda = \text{cc. of AgNO}_3 \text{ used (minus control)}$$

Procedure for Chlorides in Urine

Place 5 cc. of urine into each of two small beakers. Add to each beaker about 0.3 gm. CaCO_3 and 0.5 cc. of sodium chromite indicator. To one beaker which is to be used as a control for the end point add one drop of the AgNO_3 standard from a burette and record the amount. Into the second beaker run in AgNO_3 standard until a change in color is produced similar to the one in the control. Subtract from the last reading the recorded reading of the control. This is the amount of silver nitrate used up to precipitate all the chlorine from the 5 cc. of urine.

Calculation of Results

$$\lambda \times 10 = \text{mg. NaCl in 100 cc. of urine}$$

$$\lambda = \text{cc. of AgNO}_3 \text{ used (minus control)}$$

(If the urine is alkaline acidify it with dilute acetic acid so that it is slightly acid to litmus paper before adding the CaCO_3 .)

The accuracy of this method was checked up by adding known amounts of sodium chloride to various portions of a sample of blood and the total amounts of chlorine determined as described in the method. To a series of six flasks were added 2 cc. of blood to each. This blood was previously found to contain 437 mg. of NaCl per 100 cc. A 0.1 per cent solution of sodium chloride was added in increasing amounts starting with 1 cc. in the first flask and ending with 10 cc. in the sixth flask. The proteins were then precipitated with tungstic acid the bloods being diluted 1 to 10. Chlorine determinations were then done on 5 cc. of each filtrate. As is shown in Table I the added chloride was recovered quantitatively.

TABLE I

SAMPLE	NaCl (0.1%) ADDED	TOTAL NaCl (THIOETHANOL) IN 0.5 CC. BLOOD	TOTAL NaCl FOUND
1	1 cc.	245 mg.	2458 mg.
2	2 cc.	248 mg.	2681 mg.
3	3 cc.	293 mg.	2937 mg.
4	5 cc.	343 mg.	3441 mg.
5	7 cc.	393 mg.	3939 mg.
6	10 cc.	463 mg.	4682 mg.

The method was also checked up gravimetrically on eight samples of urine. The gravimetric procedure was done as follows: 50 cc. of urine was made acid with 1 cc. of concentrated HNO_3 and an excess of silver nitrate solution was added with constant stirring. It was heated cautiously to boil

TRANSACTIONS

Minutes of the Fifth Annual Convention, American Society of Clinical Pathologists—Dallas, Texas

THE proceedings were held in the ball room of the Baker Hotel, Dallas, Texas, April 15, 16, and 17, 1926

The convention was called to order by President Frederic E. Sondern. President Sondern appointed Dr. H. J. Corper Secretary pro tem.

Dr. Herman Spitz read the proposed changes in the constitution and by law to be adopted at the Executive Session.

Dr. Wm. H. Mounisund delivered a welcome address to the Society.

President Sondern appointed the following members to serve as a Nominating Committee: Dr. Wm. H. Mounisund, Dr. C. W. Maynard, and Dr. C. E. Roderick.

Dr. Paul Roth made a motion that a telegram be directed to Dr. Ward Burdick, Denver, Colorado, expressing to him the Society's best wishes for an early recovery. Motion carried.

The reading of papers on the regular scientific program followed.

"Hemoglobin and Erythrocytes in the South" by Dr. Leon S. Lippincott. Discussed by Dr. Carl L. Spohr, Dr. E. F. Cooke, Dr. Herman Spitz, Dr. Paul Roth, Dr. Frederic E. Sondern. Closed by Dr. Lippincott.

"A Combined Diluting and Staining Fluid for Differential Leucocyte Count in the Counting Chamber" by Dr. Daniel Nicholson. Discussed by Dr. A. H. Sanford, Dr. B. F. Stout, Dr. C. E. Roderick, Dr. F. W. Hartman, Dr. R. E. Myers, Dr. O. Lowy, Dr. Philip Hillkowitz. Closed by Dr. Nicholson.

"Sickle Cell Anemia" by Dr. G. S. Graham. Discussed by Dr. F. E. Sondern, Dr. B. F. Stout, Dr. Leon S. Lippincott. Closed by Dr. Graham.

"A Photographic Method for Counting Blood Cells" by Dr. A. H. Sanford. Discussed by Dr. H. J. Corper, and Dr. Wm. G. Epton. Closed by Dr. Sanford.

"A New Mechanical Principle for Automatic Pipettes and Blood Transfusion" by Dr. Frank W. Hartman. Discussed by Dr. C. E. Roderick, and Dr. A. H. Sanford.

The meeting was then adjourned.

Afternoon Session, April 15, 1926, 2 P M

Meeting was called to order by Dr. Sondern and scientific program continued.

"Determination of Sugar in Normal Urine" by Dr. Mark R. Everett. Discussed by Dr. Wm. G. Epton, and Dr. Wm. Taylor Cummins. Closed by Dr. Everett.

"The Glucose Tolerance Test" by Dr. W. B. Lewis. Discussed by Dr. Carl Spohr, Dr. Wm. G. Epton, Dr. A. H. Sanford, and Dr. F. W. Hartman. Closed by Dr. Lewis.

"A Study of the Pigment in Addison's Disease" by Dr. Carl L. Spohr, and Dr. Robert A. Moore. Discussed by Dr. Wm. G. Epton.

"Ochronosis" by Dr. Ernest Scott and Dr. Robert A. Moore. Paper read by Dr. Carl Spohr.

"Clinical Results with Pathogen" by Dr. Otto Lowy. Discussed by Dr. George T. Caldwell. Closed by Dr. Lowy.

"Pathology of Experimental Pyocyanus Keratitis" by Dr. F. W. Hartman and Edna Jackson.

President F. E. Sondern appointed a pro tem Board of Censors consisting of Dr. A. H. Sanford, Dr. W. W. Coulter, Dr. H. J. Corper.

Meeting adjourned.

Morning Session, April 16, 1926, 9 A M

Scientific program continued.

"Frozen Sections, Their Place, Value, and Methods" by Dr. L. A. Turley. Discussed by Dr. Michael G. Wohl, Dr. Frank W. Hartman, Dr. F. A. Hecker, Dr. Philip Hillkowitz, Dr. Herman Spitz, Dr. Edward F. Cooke, and Dr. Leon S. Lippincott. Closed by Dr. L. A. Turley.

'Laboratory Examinations Necessary and Unnecessary' by Dr George L Schadt
Read by title

'The Integration of Hospital Laboratory Work' by Dr Philip Hillkowitz Discussed
by Dr O Lowy

'The Cytomorphosis of the Tubercle Bacillus' by Dr H J Corper Discussed by Dr
Janet Caldwell Closed by Dr Corper

'Oxygenotherapy' by Dr Paul Roth

'The Treatment of One Hundred and Twenty Five Cases of Acid Intoxication with
Buffer Solutions' by Dr F A Hecker

Afternoon Session April 16 1926 2 P M

Scientific program continued

'Intestinal Amebiasis from the Pathologic Standpoint as Related to the Clinical with
Preliminary Report of X Ray Studies of Early Cases' by Dr J M Feder Discussed by Dr
Isaac J Jones Dr Kenneth M Lynch Dr A H Sanford Dr W S Thomas Dr T C Terrell
Closed by Dr Feder

'The Hirsch Abderhalden Test' by Dr F E Sondern Discussed by Dr Wm G
Exton, Dr Otto Lowy Dr Mark R Fverett, and Dr Michael G Wohl Closed by Dr Sondern

'Comparison of Kolmer and Kahn Tests' by Dr C E Roderick Discussed by Dr T
C Terrell Dr A H Sanford Dr A S Giordano Dr F A Hecker Dr George T Caldwell
Dr J M Feder, Dr F W Hartman Dr H J Corper and Dr B F Stout Dr Roderick
closed the discussion

Meeting was then adjourned

Morning Session, April 17 1926 9 A M

Business session called to order by President Sondern

The reading of the minutes of the last meeting was dispensed with

Dr Philip Hillkowitz gave the report of the Committee on Commercial Exhibits for Dr
Ward Burdick and a motion was made and carried that it be accepted

The report of the Publication Committee was given by its Chairman Dr Philip Hillko
witz, who said that present arrangements for our official organ were not ideal and that even
tually there would have to be a publication controlled entirely by the American Society of
Clinical Pathologists of which the committee realizes fully the difficulties involved in the way
of financing managing and editing a proposition by Dr Herman Spitz to be brought before
the Society a little later A motion was made that the report be accepted and the thanks of
the Society be extended to the Publication Committee Motion carried

Report of the Committee on Methods of Transportation of Laboratory Specimens was
made by Dr Herman Spitz Chairman, who said that no leaflet had been prepared owing to
pressure of other business but that the post office department issued a set of rules governing
this matter These rules and regulations were received and published in the *Bulletin of the
American Medical Association* of February xxi p 62 A permit for sending and receiving
specimens through the mails may be obtained from the postmaster Motion made and car
ried that report be accepted

Report of the Committee of Laboratory Standardization was made by President Sondern
for Dr Thaddeus Walker, Chairman of that Committee who was absent Dr Sondern said
that the Committee had been inactive because they deemed it advisable to wait and see what
the American Medical Association did in the matter Motion carried that report be adopted

The report of the Executive Committee was given by Dr Herman Spitz Chairman, who
gave the financial report and stated that the books of the Secretary were found to be correct
This report was accepted by motion of the Society

The matter of a new code of ethics was taken up and the decision was reached that the
present code was sufficient Report accepted

Dr Spitz attended as representative of American Society of Clinical Pathologists, and
read papers at two meetings of the American College of Surgeons Report accepted

The report on the Committee on State Laboratories by Dr Herman Spitz was made he
gave various illustrations and records showing the tremendous amount of laboratory work
done by State Laboratories in competition to practicing clinical pathologists Discussion
followed Report of Executive Committee accepted by Society

Dr Herman Spitz read the revised constitution and by laws and they were adopted.

Dr H J Corper gave the report of the Service Bureau Committee for Dr Ward Burdick, telling of the beginning of this feature of the Society. A motion was carried that this report be accepted and the thanks of the Society be extended to the Secretary for his work.

The matter of the Registration of Technicians was discussed and a motion carried that the President appoint a committee to investigate this matter and report to the Society at the next annual meeting.

The election of new members followed. Upon recommendation by the Board of Censors pro tem the following were elected to active membership:

Dr Oliver W Lohr, Saginaw, Michigan
 Dr Leonard W Larson, Bismarck, N D
 Dr Charles F Carter, Dallas, Texas
 Dr C Y White, Philadelphia, Pa
 Dr Kenneth M Lynch, Dallas, Texas
 Dr N W Loud, Colorado Springs, Colorado
 Dr Oscar G Costa, San Juan, Porto Rico
 Dr Nathan Rosenthal, New York City, N Y
 Dr Wm Taylor Cummins, San Francisco, California
 Dr Wm J Muzzy, El Reno, Oklahoma
 Dr Philip B Matz, Washington, D C
 Dr Wm McKee German, Grand Rapids, Michigan
 Dr Joseph P Garen, Olean, N Y
 Dr Reed Rockwood, Baltimore, Md
 Dr C H Manlove, Portland, Oregon
 Dr G W Millett, Portland, Oregon
 Dr Carl Boettiger, Astoria, Long Island

To associate membership:

Dr Mark R Everett, Norman, Oklahoma
 Dr Isaac J Jones, Little Rock, Arkansas

Dr John A Kolmer suggested that a book of approved methods be published under the auspices of the American Society of Clinical Pathologists. A motion was made that a Research Committee be appointed by the President to investigate the question and study the advisability of such a publication and the ways and means of bringing this about, which committee was to have power to proceed. Motion carried.

The matter of a new journal, published and controlled entirely by the Society was opened. Dr Herman Spitz presented a proposition for the consideration of the Society which was referred to the Executive Committee with power to act.

It was moved that a Committee be appointed by the President to investigate the matter of bringing the views of the Society forward by various ethical methods to be determined by this committee which was given power in the matter.

The election of officers for the ensuing year resulted as follows:

President Elect	Dr A H Sanford, Rochester, Minnesota
Vice President	Dr J H Black, Dallas, Texas
Secretary Treasurer	Dr Ward Burdick, Denver, Colorado

Executive Committee

Dr Philip Hillkowitz, Chairman, Denver, Colo
 Dr Wm Carpenter MacCarty, Rochester, Minn
 Dr F W Hartman, Detroit, Mich
 Dr John A Kolmer, Philadelphia, Pa
 Dr W W Coulter, Houston, Texas
 Dr Herman Spitz, Nashville, Tennessee

Board of Censors

Dr George Ives, Chairman, St Louis, Missouri
 Dr Ernst A Victors, San Francisco, California
 Dr Paul Roth, Battle Creek, Mich
 Dr W F Thomson, Beaumont, Texas
 Dr Robert A Kilduffe, Atlantic City, New Jersey
 Dr H R Mills, Tampa, Florida

Dr Wm G Extou, President for 1926-7 was escorted to the Chair and the meeting was adjourned.

DR. WARD BURDICK, SECRETARY

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A KILDUFFE MD ABSTRACT EDITOR

Clinical and Experimental

PERTUSSIS Significance of the Blood Chemical Changes in Pertussis Regan, J C and Tolstouhov A. Jour Am Med Assn, April 10 1926 lxxvii 1166

A study of 111 cases ranging in age from four months to twelve years, 78 per cent being over three years of age

A total of 682 blood chemical analyses performed in cases of pertussis have given the following results

1 There is a diminution of the total inorganic phosphorus associated with a lowering of the hydrogen ion concentration of the blood, while the plasma bicarbonate remains within normal limits

2 These changes occur early in the disease, appearing in the case of the inorganic phosphorus in the catarrhal stage

3 Both alterations are well developed, especially the change in phosphorus during the first few weeks of the paroxysms and show a certain degree of parallelism in their course which signifies a close interrelation

4 In moderate and severe cases treated with alkalis the inorganic phosphorus rises steadily from the third week, while in untreated cases of the mild type the rise does not begin until the sixth week. The same is true in a less decided way of a P_H value before as compared to those during and after treatment

5 The diminution of inorganic phosphorus bears no relation to age but only to the stage of the disease, and for reasons mentioned in the text has no underlying rachitic basis

6 The calcium content, while exhibiting slight mobility as the result possibly of shifting of calcium in connection with the characteristic phosphorus and P_H alterations, has no constant alterations of a distinct type

7 These changes indicate an acidosis of an uncompensated type (type 6 Van Slyke) which has as a cause the accumulation or increased concentration of free carbon dioxide in the blood. This laboratory observation is easily correlated with several of the symptoms so prominent in pertussis—the paroxysms, the vomiting, parenchymatous emphysema and convulsions

8 The vomiting of the disease may be a compensatory mechanism adopted by the body to eliminate acid in an attempt to maintain a normal acid base balance

9 This contention of an uncompensated acidosis is further substantiated by the effects of the disease of alkali therapy

10 Alkalis administered early appear usually to abort the disease and associated with the cure is a rapid rise of inorganic phosphorus and a change in P_H of the blood, while if given late, cure supervenes in a relatively short period

Conclusions There occurs in pertussis an uncompensated acidosis which is intimately connected with the pathogenesis of the paroxysms. If the acid base unbalance is corrected the clinical symptoms are quickly ameliorated, and the organism returns to normal

EDEMA The Cutaneous Test for a Hydrophilic Condition Labbe M. Presse Med., Paris, May 22, 1926 xxxiv, 641

When small quantities of physiologic salt solution are injected into the derma of an edematous patient the small blister thus produced disappears in a few minutes while it remains for more than an hour in a normal individual. Interesting experiments have been performed with this fact as a basis

The injection cannot be considered as properly effected unless it produces a small round local elevation, snow white and showing plainly the pores of the skin. A small erythematous zone surrounds this elevation

The time elapsing before the disappearance of this blister varies for different pathologic conditions, and its evaluation throws light on the more or less accentuated hydrophilic condition of the tissues and humors.

The slight traumatic elevation produced by the introduction of the needle must be distinguished from that due to the saline injection. The former may persist after the latter has completely disappeared.

It has been noted that in edematous patients, the more accentuated the edema, the more rapid the disappearance of the blister, and inversely. In incipient edema a reduced period of disappearance precedes clinical signs of edema by several days.

The Barthelémy syringe is to be recommended, as well as the use of as fine a needle as possible.

In ascitic patients, the time of disappearance diminishes as the ascitic fluid increases. The shorter the period of disappearance, the greater the increase in the ascites.

After serious hemorrhage the time of disappearance increases with the reestablishment of the bulk of the blood stream. Thus the diminution of the time of disappearance produced by intradermal injection of a saline solution indicates a thirst for water in the tissues. This test has an important bearing on the study of the hydrophilic condition of the tissues and humors, and hence that of the pathogenicity of the edema present.

Edematous plasma was injected into three normal subjects and the resulting blister did not disappear before the expiration of the time previously required for the disappearance of the blister following injection of the salt solution, that is, from one to one and one half hours. When injected into a patient suffering from Laennec's cirrhosis with ascites, the time of disappearance was reduced to fifteen minutes.

Upon injection of normal plasma into three normal subjects, the time of absorption varied from fifteen to seventy five minutes.

Thus the test gave the same results whether made with normal plasma, edematous plasma or physiologic salt solution, showing that the rapidity of the time of absorption is independent of the liquid injected, and depends entirely on the hydrophilic powers of the tissues and humors.

Diabetic patients, even where there are no clinical indications of edema, have a particular tendency to retain water in their tissues, nor does insulin treatment restrict this predisposition. Many diabetic patients are in a preedematous state, evidenced by the peculiarly elastic and soft consistency of the tissues noticeable upon palpation. The cutaneous test corroborates such evidence as the time of disappearance is always noticeably shortened.

BLOOD SEDIMENTATION The Relation of the Erythrocyte Sedimentation Reaction to the Ability of Flocculation of the Plasma and Serum, Rubin, E. H. *Arch. Int. Med.*, June, 1926, *LXXVI*, No. 6, p. 848.

Depending on the toxicity of a disease process, a proportional increase in the erythrocyte sedimentation reaction and the ability of flocculation of the plasma and serum, as determined by the Frisch and Starlinger, Gerloery and Matefy reactions, was found. The Daranyi reaction was negative in forty cases studied.

Because of its greater simplicity, accuracy and wider range of readings the sedimentation reaction seemed the most practical test to use clinically.

BLOOD SUGAR A Comparison of the Folin-Wu and the New Benedict Method for Sugar in Blood and Cerebrospinal Fluid, Lyttel, J. D., and Hearn, J. E. *Jour. Biol. Chem.*, June, 1926, *LXXVI*, No. 3, p. 751.

Simultaneous blood and cerebrospinal fluid sugar determinations were made on twenty six cases by the Folin-Wu method and by Benedict's new method.

In the majority of cases the Folin-Wu method gave higher values than did the Benedict method.

In the blood the average difference by the two methods was 12.4 mg., with 17.5 per cent of the cases showing practically no difference.

The nonprotein nitrogen content of the blood had no relation to differences in sugar content by the two methods

In the cerebrospinal fluid the average difference was 0.1 mg with 52.6 per cent of the cases showing practically no difference

The interfering substance or substances are not present in the cerebrospinal fluid so constantly or in such large amounts as in the blood

The protein and nonprotein nitrogen content of the cerebrospinal fluid has no relation to differences in sugar content by the two methods

EDEMA Intradermal Salt Solution Test in Normal and Toxemic Pregnancies Lash A. F Surg, Gynec and Obst July 1926 p 40

The technic of McCuro and Aldrich was employed as follows 0.2 cc of an 0.8 per cent aqueous solution of sodium chloride was injected intracutaneously under aseptic conditions A duplicate injection was made about 2 centimeters from the first as the disappearance of the depression between the two wheals was an aid in determining the end point The flexor surface of the forearm and the medial surface of the leg were the sites selected for the injections The disappearance time was the time taken for the elevations or wheals to disappear as determined by palpation The frequency of observations depended on the character and course of the case thus, in the normal pregnant women one careful test was considered sufficient while in the abnormal tests were performed at intervals of several hours, days, or weeks In the normal women a five to ten minute variation in disappearance time was considered of no significance as the end point was not always well defined in this group, although definite in the toxemic women

A normal group of 47 pregnancies was studied (20 white and 27 colored 15-36 years of age, 26 I para, 21 multiparae urine and blood pressure normal)

A group of toxemic cases was also studied (17 with convulsions 29 without)

The following conclusions were drawn

The disappearance time of intradermally injected salt solution in normal pregnant women is longer in the negro than in the white This racial difference can possibly be explained by the thicker skin in the negro (Unna, 8)

The women with the toxemias of pregnancy show definitely decreased disappearance time, more marked in those with convulsions The degree of decrease in the disappearance time varies directly with the degree of severity of the toxemia increasing with the general clinical improvement

The same factor or group of factors, that produces the edema, hypertension and albuminuria in the late toxemias of pregnancy, apparently produces the condition in the tissues which give a reduced disappearance time Hence the intradermal salt solution test may prove a valuable aid in diagnosis and prognosis of these conditions

The use of the test routinely during the later months of pregnancy may prove of value in determining the oncoming of a toxemia earlier than by other methods now available

THYROID DISEASE Diagnostic Value of the Kottmann Reaction in Thyroid Dysfunction Katayama I Am Jour Med Sc July 1926 clxxx No 1 p 84

A study of 101 cases with the conclusions following

It is generally conceded among physiologists and clinicians that the determination of the basal metabolic rate is the most dependable laboratory index of thyroid activity In creased thyroid secretion produces a lowering of the tolerance for carbohydrate, but there are numerous other conditions in which the tolerance for carbohydrate may be diminished Hence the occurrence of high blood and urine sugar curve after the ingestion of glucose is not in itself indicative of hyperthyroidism In hypothyroidism, however, the blood and urine sugar curves after glucose furnish information concerning a phase of carbohydrate metabolism which is not gauged by the basal metabolic rate

The basis of the Kottmann reaction is obscure, and hence it is difficult to say in what manner thyroid activity influences it Such a reaction can only be accepted with skepticism From the data reported in this paper it is evident that the results of the Kottmann reaction

are not in accord with the basal metabolism or the glucose tolerance. The retardation of the reduction of the silver iodide to silver occurs in many and various conditions patently not due to hyperthyroidism. Its diagnostic value in detecting hyperfunction or hypofunction of the thyroid is very dubious. It cannot be accepted as a substitute for the determination of the basal metabolic rate or of glucose tolerance.

ECLAMPSIA The Blood Chemistry in Eclampsia, Stander, H. J., and Radelet, A. H. Bull. Johns Hopkins Hosp., June, 1926, LXXVIII, No. 6, p. 423

Zweifel, in 1904, showed that in the urine of eclamptic women the urea nitrogen is lowered and the ammonia nitrogen raised. From this, he reasoned that there ought to be an increase in some acid in the blood, and that it was probably in lactic acid. He accordingly analyzed the urine and blood for that substance, precipitating it as zinc lactate. He reported eight eclamptic cases in which he was able to show the presence of lactic acid in the blood. From one of the patients, he obtained two specimens of blood, the first showing lactic acid, while the second did not, this second sample of blood having been taken about five hours after the last convulsion. He furthermore stated that in normal pregnancy he was usually unable to demonstrate the presence of lactic acid. It might be added that Futh and Lockemann demonstrated the presence of lactic acid in the cerebrospinal fluid of eclamptic patients. The authors' figures on the other hand, show that, in the blood of normal pregnant women, the lactic acid is within the limits for the normal nonpregnant person, and that in eclampsia there is a pronounced increase, amounting to 200 per cent or more above the normal.

A sample taken from an epileptic pregnant woman a few minutes after a violent convulsion showed only a slight increase in lactic acid, (45.32 mg.) so that the increase is not markedly influenced by the muscular activity due to convulsions.

Neither does it seem to be due to interference with the excretion of lactic acid.

The authors suggest three possibilities to explain the high lactic acid content of the blood: (1) An abnormally great amount of carbohydrate metabolized, (2) a disturbance or slowing down of the "lactic acid to glycogen" step in the carbohydrate chain, (3) lactic acid production from sources other than carbohydrate, such as protein.

ANACIDITY A Statistical Study of the Diagnostic Value of Acidity, Hartman, H. E., and Sager, W. W. Med. Jour. and Rec., New York, July 21, 1926, LXXIV, 96

A study of 492 cases studied by fractional gastric analysis from which it is concluded that the chances for and against the presence of pernicious anemia, gall bladder disease, or carcinoma, in a case of anacidity, are about even, the chances of either pernicious anemia or carcinoma being present are about one in three.

Carcinoma is rare when free hydrochloric acid is present.

PERNICIOUS ANEMIA The High Color Indices in Pernicious Anemia, Komiya, E. J. J. hematol., May, 1926, XXII, 201

Komiya recalls that while the high color index so frequently noted in pernicious anemia has been ascribed to the large number of megalocytes present, this conclusion has been disputed.

He reports his studies in this disease leading to the conclusion that, in pernicious anemia, not only the megalocytes but all the larger erythrocytes, except those showing polychromasia, carry larger amounts of pigment and that the increase of the color index is due to a general hyperchromia of the erythrocytes.

The hemoglobin content of embryonic blood is relatively high, but the erythrocyte count is less than in the adult. As a result the embryonic color index is high due to the presence of abnormally large red cells as shown by a high volume index. The so-called normoblasts of embryonic blood correspond to the macroblasts or macrocytes of adult blood.

Komiya states that in pernicious anemia the blood formation is similar to that in embryonic life and that the increased color index is caused, not only by the megalocytes in Ehrlich's sense, but also by the macrocytes which are an even more important factor.

Laboratory Technic

HOOKWORM The Place of the Smear in Hookworm Diagnosis Hausheer W C and Herrick C A *Am Jour Hyg*, July (Supplement) 1926 vi 136

The smear method is diagnostically accurate if the intensity of infestation is such that 500 or more ova are present per gram of feces

The smear method, if only two slides are examined for a negative, will fail in a certain percentage of cases yielding between 300-500 per gram of stool

If less than 300 ova are present per gram of stool the smear is highly inaccurate

When the above factors are taken into consideration the smear method may be used with confidence, it is simple rapid and still holds a valuable place in the fecal examination for hookworms.

HOOKWORM Evaluation of the Methods of Stoll and Lane in Light Hookworm Infections and Accuracy in Diagnosis of the Willis Floatation Method Hausheer W C Herrick, O A and Pearse A. S. *Am Jour Hyg*, July (Supplement), 1926, vi, 118

In a series of seventy carefully studied stools the regular Stoll technic (large drop) will detect cases having as low as fifty eggs per gram by Lane count, if not less than two slides are examined

Lane's method pushed to finality demonstrated 68.3 per cent of the ova indicated present by the regular drop dilution egg count, and diagnostically showed every stool positive

The small drop Stoll technic demonstrated 94.5 per cent of the ova, shown present by the regular dilution counts. The small drops failed diagnostically (two slides only being counted from each tube) in 75 to 80 per cent of cases having less than 200 ova per gram, but showed high diagnostic efficiency in the higher counts

An analysis is presented of the Willis floatation method, and its efficacy, when properly handled, is emphasized. It would appear to be as accurate as Lane's direct centrifugal floatation when certain details in its use are observed

The floatation method as practiced by the authors, is as follows

1 The specimen of feces is thoroughly mixed in the container

2 A small amount (1 to 2 gm) is *thoroughly* comminuted with salt solution having a specific gravity of 1.150 to 1.200

Care in the comminution of feces and salt solution and in the use of a solution of proper density, are essential factors for success

3 The container is then filled to the brim with additional saline solution

4 A glass slide, of such size that it will more than cover the container is placed thereon and allowed to stand for ten to fifteen minutes

As ova are destroyed by concentrated saline in an hour this period should not be longer than thirty minutes.

5 Remove the slide carefully without losing the adherent fluid quickly invert, and examine under low power

CULTURE MEDIA The Exudate from Nutrient Agar Slants—The So Called Water of Condensation Healy D J *Jour Bacteriol* September 1926 xii, No 3 p 179

As is well known, a variable quantity of liquid collects in nutrient agar slant tubes, a condition which does not occur in the case of nutrient gelatin slants. For many years this liquid has been known as the water of condensation.

As many microbes grow more freely in this so called water of condensation than they do on the surface of the agar slant or in nutrient broth, it seemed desirable to determine its composition

As a result of his analysis the author concluded that the so called water of condensation which collects in nutrient agar slant tubes is an exudate from the nutrient agar, possessing nutrient substances suitable for sustaining bacterial growth

HOOKWORM Estimation of the Number of Hookworms Harbored by the Use of the Dilution Egg Count Method, Hill, R B *Am Jour Hyg*, July (Supplement), 1926, vi, 19

In order to check the suggestion of Stoll that the factor 44 represents the average egg output per gram of feces per female hookworm, and that such a factor can be used to estimate the number of parasites when only eggs per gram are known, the total egg output of 93 heavily infested cases was estimated for two, or three days, and compared with the number of worms recovered after treatment to a cure.

A positive correlation was found between each of the items the total daily egg output, the number of eggs per gram, basis formed, and the number of female hookworms harbored.

The calculations resulted in finding that the factor 183 represented the number of eggs per gram per female in this series. This factor, when applied to the egg counts in the series, gave a close estimate of the number of female hookworms harbored, but when applied to other groups was usually too low. Stoll's proposed factor of 44 was too high.

It was found that feces recovered could be classified as formed, soft, or mushy, and the average amounts of these classes, per day, were 147, 226, and 314 grams respectively, a ratio of approximately 1 : 1.5 : 2. It is suggested that, for comparison, all counts per gram be reduced to the basis of formed stools by the use of the proper factor.

It is suggested that as the number of worms harbored increases, the egg output per worm decreases.

IMMUNITY The Role of the Reticulo Endothelial System in Immunity II The Complement Titer After Blockade and the Physiologic Regeneration of the Reticulo Endothelial System as Measured by Reduction Tests, Jungeblut, C W, and Berlot, J A *Jour Exper Med*, June, 1926, lxxv, No 6, p 797

Intravenous injections of India ink into guinea pigs caused a decided drop in the complement titer which set in as early as fifteen minutes after the injection, but did not reach its minimum for three hours. This drop was followed by a return to normal within the first twenty-four hours following the injection.

India ink mixed in vitro with guinea pig serum adsorbs the complement almost immediately to its full extent.

By means of reduction tests (methylene blue and nitroanthraquinone) it was shown that the respiration of the cells of the liver and spleen of guinea pigs was markedly impaired for the first eight hours, following an intravenous injection of ink. Evidence of a return to normal functional vitality, however, became apparent by the end of the first day after the injection.

GONOCOCCUS Study of Agglutination of the Gonococcus in Man, Jenkins, C E *Brit Med Jour*, July 3, 1926, xi, 3417

Agglutinins for the gonococcus are not produced in man when the infection is limited to the genitourinary system. In generalized infections, such as arthritis, the production of agglutinin is so slight and uncertain as to render such a test useless for diagnosis.

STAINING TECHNIC A New Staining Dish, Kracke, R R *Jour Am Med Ass*, July 3, 1926, lxxvi, 29

The entire apparatus is constructed in three parts, the support, outside container, and slide rack. The stand is made of rigid black enameled iron with a row of ten micro burners which supply an even and adequate distribution of heat to the stain within and is so arranged that the container can be moved forward or backward to facilitate draining from the outlet petcock on the bottom.

The outer container and slide rack are both constructed of monel metal, which is noncorrosive and extremely durable, insuring long continued usage. The outer container consists of a metal rectangular box of outside dimensions of approximately 14" by 12" by 10".

by 10 inches and a capacity of 600 cc when empty and 400 cc when filled with slides. At one end near the top is an inlet to which a water hose can be connected and at the opposite end on the bottom an outlet petcock through which the stain and water can be drained. The bore of the inlet and outlet is of sufficient caliber to insure a rapid inflow and a rapid outflow of stains or reagents the dish emptying in about twenty seconds with the petcock open fully.

The slide holder fits snugly into the outer container with sufficient space allowed so that it can be lifted and replaced easily facilitating the thorough washing of slides by repeated immersion. It consists of a framework containing fifty slots for that number of slides and a bottom slide rest, the construction being such that the rack filled to its full capacity can be vigorously shaken to remove excess water or stain.

Technic for use. The required number of specimens to be stained are placed within the rack and then placed into the outer container. The first stain to be used is then poured into the end space provided for that purpose the stain flowing beneath the slides and welling up between them. Sufficient stain is poured in to cover the slides to the highest level of the smears the upper portion of the slides remaining free from stain.

After the stain has acted for the required time it is drained back into the stock bottle through the lower petcock. The other stains and reagents are poured in and drained off in a similar manner. Whenever water is indicated in the process it is allowed to flow in through the upper petcock and drained off or a continuous flow can be maintained when thorough washing is desired.

After completion of the staining process with tissue sections they can be removed one by one and mounted or if suspected tuberculous sputum is being stained the entire rack can be removed and the slide allowed to dry in the air. When staining tuberculous sputum the same procedure is carried out the necessary heat for steaming with carbolic fuchsin being provided by the burners beneath.

TUBERCULOSIS. A Method of Producing Defatted (Nonacid Proof) Living Cultures of B. Tuberculosis with a Preliminary Report on the Same as an Immunizing Agent, Whitman, B. C. and Chambers, K. L. Colorado Med. April 1916 vol. 118

The method depends upon the use of the following culture medium.

The ends of long bones or the vertebra of cattle rich in marrow are stripped as clean as possible of fat tendon ligament and muscle, and broken up with a hammer and chisel into pieces the size of an English walnut or better ground in a green bone grinder such as is used by poultry men. It is not necessary to exclude rigidly portions of the bone from the shaft side of the epiphyses but the red marrow gives better results than the yellow marrow and should make up as much of the material as possible. Weigh piece in a suitable vessel and boil over the open flame for an hour or more. Decant the fluid and when cold remove the thick layer of fat which collects at the surface. Add water to make the amount equal in cubic centimeters to the weight of the bone in grams. Add 0.5 per cent salt 1 per cent Witte peptone and 1.5 to 2 per cent powdered agar, heat to dissolve these and without filtering tube and autoclave. All the lots we have so far made up have been neutral or very slightly acid to litmus without adjustment. As the tubercle bacillus does best on media somewhat more acid than that best adapted for general purposes, the reaction above mentioned serves very well.

On this medium the tubercle bacillus is nonacid fast the property being developed after transfer to egg media or media containing lecithin.

A small series of guinea pigs was immunized with such cultures and later inoculated with the authors believe encouraging results.

Their conclusions follow. On agar medium made from bone instead of meat infusion the tubercle bacillus grows wax free (i.e. nonacid proof) in the first generation.

Vaccines prepared by the customary method from such wax free cultures of an old greatly attenuated strain of human type bacillus afford practically complete protection to guinea pigs against subsequent injection of overwhelming doses of virulent bovine bacilli.

This protection lasts for about one year, and no doubt for a longer time though in somewhat lower titer.

The same vaccine seems to have considerable curative value in established tuberculo is

The wax free organism may prove to be a useful antigen for complement fixation tests

It is to be hoped that improvement in the method of preparing and using the vaccine may increase its usefulness. Possibly the use of living wax free bacilli as a vaccine may be found to be both safe and advantageous

BLOOD CALCIUM A Colorimetric Method for the Estimation of Blood Calcium, Roe, J H, and Kahn, B S Jour Biol Chem, March, 1926, LVII, 585

Reagents

Trichloroacetic acid 20 per cent

Phenolphthalein 1 per cent

Sodium hydroxide, calcium free 20 per cent

Trisodium phosphate 1 per cent

Sulphuric acid 5 per cent

Sodium or ammonium molybdate 5 per cent

Standard phosphate solution Dissolve 4.394 gm of pure dry monopotassium phosphate in 1,000 cc of water. One cc contains 1 mg of phosphorus. Preserve this stock solution with chloroform

Five cc of this solution diluted to 1,000 cc with phosphate free water is the solution for calcium estimation, 10 cc containing 0.05 mg of phosphorus equivalent to 0.097 mg of calcium phosphate

Hydroquinone bisulphite reagent 30 gm sodium bisulphite and 1 gm hydroquinone (highest purity), dissolved in 200 cc phosphate free water. Calcium free filter paper

Place 2 cc blood serum in a small flask and add 4 cc of distilled water and 4 cc of 20 per cent trichloroacetic acid. Mix thoroughly, allow to stand ten minutes, and filter through a double acid washed calcium free filter paper. Transfer 5 cc of the trichloroacetic filtrate to a 15 cc conical centrifuge tube which has been thoroughly cleaned by immersion in bichromate sulphuric "cleaning solution" for several hours. Place one drop of 1 per cent phenolphthalein in the tube and add, a drop at a time, 20 per cent calcium free hydroxide until a definite pink color is obtained. Add 1 cc of 1 per cent trisodium phosphate. Swirl the tube until thoroughly mixed, cork, and set aside for one hour.

After one hour's standing, centrifuge for three minutes. Decant carefully the supernatant fluid from the calcium phosphate precipitate. Place the inverted tube upon a pad of filter paper to drain for two or three minutes, then wipe away adherent solution from the mouth of the tube with a clean cloth or paper. Wash twice with 5 cc portions of 50 per cent alcohol made faintly alkaline to phenolphthalein with a few drops of calcium free alkali. In washing, the mat of calcium phosphate in the bottom of the tube must be thoroughly broken up with a glass stirring rod, and the process of centrifuging, decanting, and draining the tube should be carried out as described above. Dissolve the washed precipitate in 5 cc of 5 per cent sulphuric acid by volume (5 cc concentrated acid per 100 cc of water), and decant into a Rothberg Evans sugar tube, or a graduated test tube, wash the centrifuge tube twice with approximately 3 cc and 2 cc portions of the 5 per cent sulphuric acid, adding the washings to the graduated tube.

In a similarly graduated tube place 10 cc of standard phosphate solution containing 0.05 mg of phosphorus, and add 0.5 cc of concentrated sulphuric acid. Now add to each tube 1 cc of 5 per cent sodium molybdate and 1 cc of hydroquinone bisulphite reagent. Place the tubes in a boiling water bath for ten minutes. Remove, cool, dilute the standard to 15 cc and the unknown to a volume giving a color that will approximately match the standard (15 cc in normal bloods), and compare in a colorimeter in the usual manner.

The calcium content in milligrams per 100 cc of serum is then calculated from the formula

$$\frac{R_1}{R} \times 0.05 \times \frac{D_2}{D_1} \times \frac{60}{31} \times 100$$

in which R_1 is the reading of the standard, R , the reading of the unknown, D_1 , the dilution of the standard, and D_2 , the dilution of the unknown.

The Journal of Laboratory and Clinical Medicine

VOL. XII

ST. LOUIS, MO., APRIL, 1927

No. 7

Editor in Chief WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	CINCINNATI
PAUL G. WOOLLEY, M.D.	LOS ANGELES
J. J. R. MACLEOD, M.B.	TORONTO
W. C. MACCARTY, M.D.	ROCHESTER, MINN.
GERALD B. WEBB, M.D.	COLORADO SPRINGS
VICTOR C. MYERS, Ph.D.	IOWA CITY
RUSSELL L. HADEN, M.D.	ROSEDALE, ILL.
JOHN A. KOLMER, M.D.	PHILADELPHIA
ROBERT A. KILDUFFE, M.D.	ATLANTIC CITY, N. J.
A. PARKER HITCHENS, M.D.	MANILA, P. I.
DEAN LEWIS, M.D.	BALTIMORE
GEORGE B. HERRMANN, M.D.	NEW ORLEANS
HAROLD D. CAYLOB, M.D.	ROCHESTER, MINN.

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo. as Second Class Matter

EDITORIALS

New Views on Carbohydrate Metabolism

THE researches of the past few years dealing with the chemistry of muscular contraction have led to the conclusion that carbohydrate and thus alone is oxidized in the process. The evidence for this belief is mainly that the volume of CO₂ produced during the entire process of contraction and restoration to the original state is equal to that of the oxygen consumed, the respiratory quotient is unity. This has been shown both for isolated muscle contracting outside the body and for the extra amounts of O₂ and CO₂ used by the intact animal (man) while performing muscular work. To obtain the latter values the resting (basal) amounts of O₂ used and of CO₂ expired by the animal are subtracted from the amounts used and expired not only during the exercise itself but also for such a period thereafter as is necessary to bring the values back to the basal level. Supporting evidence for the same view is also afforded by the fact that direct chemical analysis of the muscle reveals dimin-

ution in the amount of glycogen as a result of contraction and no significant change in any other organic constituent

If we accept this view it leads to the very important conclusion that proteins and fats can be used for the production of muscular energy only after they have been converted into carbohydrate. That such conversion readily occurs in the case of protein is well known as a result of studies of the metabolism in diabetes, but the same studies are usually considered also to show that no carbohydrate is derived from fat or, more correctly, from fatty acid since the glycerol portion of fat itself is readily changed into sugar. The main evidence for this belief is that the ratio between the excretion of dextrose and nitrogen in a starving, or protein-fed, animal poisoned with phlorizin remains at a constant level (1.365) from day to day. But such an animal can scarcely be regarded as strictly a diabetic one since at least two of the cardinal symptoms of this disease are missing, namely, hyperglycemia and marked ketosis. When depancreatized animals are observed, on the other hand, the D/N ratio does not, as a rule, remain constant from day to day, whereas all the symptoms of the diabetic state as observed in man are prominent. This type of evidence against the conversion of fat to carbohydrate is, therefore, inconclusive and when we remember that conversion of fat to carbohydrate can easily be shown to occur in plants and that the process is quite explicable on a purely chemical basis, the probability that fat is converted to carbohydrate in the metabolism of all animals becomes considerable.

To convert protein and fat into carbohydrate requires that more oxygen be incorporated in the molecule since the former is built up of numerous methyl (CH_3) groups and the latter of alcohol (CHOH) groups. This absorption of oxygen must consequently lead to very marked decrease in the R.Q. ($\frac{\text{CO}_2}{\text{O}_2}$). Thus, if we compare the chemical formulae of fatty acids found present in the animal body with that of a simple sugar it is clear that the quotient must fall to about 0.2 or 0.3. But actual analysis of the respired air of completely diabetic animals, in which it is believed this new formation of sugar will be proceeding at its fullest intensity, shows that the quotient is never lower than 0.66. How are we to explain this? We can do so if we assume that the oxygen absorption occurs somewhere in the body outside the muscles the partially oxidized molecule being then carried as sugar to the muscles where it is oxidized. The R.Q. of the animal as a whole will then be the algebraic sum of the low quotient of the gluconeogenic process occurring in the liver and the high one of the final oxidation process occurring in the muscles. That this *net* quotient should under certain standardized conditions remain at a constant level, as in a phlorizin-poisoned dog is no more remarkable than that the body temperature, or the blood sugar or the daily excretion of nitrogen remains constant.

According to such a view the main fault in metabolism responsible for diabetes must be excessive gluconeogenesis. This is admitted by all investigators to be the case for protein, but is denied for fat. And yet there is much to indicate that fat is also concerned. Fat metabolism certainly goes wrong in diabetes and it has been suggested that the ketone bodies are really in

products of the chemical process by which fat is converted into carbohydrate. Attempts to disprove this hypothesis by seeing whether feeding with fats will increase the sugar excretion in diabetes, are of course futile, since it is well known that fat after its absorption goes through a lengthy process before it is finally drafted to the liver to be prepared for ultimate oxidation. Experience, both in the laboratory and the clinic affords strong support to the "fat derivation" hypothesis. Thus we have observed that when depancreatized dogs treated with insulin are made fat, by feeding them with excess of carbohydrate they exhibit much more acute symptoms of diabetes when insulin is withdrawn than are observed under the same circumstances in the case of thin dogs. The hyperglycemia, ketonemia and glycosuria are all more intense, but most striking of all, the general symptoms are extremely acute and a fat animal seldom lives for more than four days after discontinuing the insulin, whereas a thin one may live several weeks.

There is therefore no irrefutable evidence against the view that fat, as well as protein, must be converted into carbohydrate in the liver before it can be utilized as fuel by the muscles. On the contrary recent results are all on its support and among these, experiments by Soskin may be of interest. It is well known that the blood sugar steadily falls when the liver is removed from the body. This has been considered to show that the liver must at least be the chief source of the blood sugar, but it has not been concluded that it is its only source. Soskin injected large amounts of epinephrin into hepatectomized dogs and subjected others to asphyxia under ether anesthesia without causing even the slightest increase in the steadily falling blood sugar and he found after death that the muscles still contained some glycogen. The glycogen of muscle cannot apparently be reconverted to sugar in the body. It can of course be thus converted *in vitro* by hydrolysis either with acid or diastase, but once it has become deposited within the living muscle it has entered an irreversible reaction which leads it through lactic acid to CO_2 and H_2O . In asphyxia as in Soskin's experiments no doubt so much of this lactic acid accumulated in the muscles that some found its way into the blood. If the liver had been present this blood lactic acid would have been converted into glucose for this process is known to occur in the intact animal, but in the liverless animal it remained unchanged and there was no rise in blood sugar. That the glycogen in the muscles should diminish as Mann and Magath have shown in the liverless animals is of course easily accounted for by the constant using up of this material.

It may be pointed out that Mukowitz has found that the respiratory quotient rises sometimes almost to unity for periods of several hours' duration after removal of the liver in dogs which are kept alive by injection of sugar. It does not rise so high without sugar injection but that it rises at all over sufficiently long periods of time to rule out any error due to a blowing off of CO_2 is supporting evidence for the above views.

Finally it may be concluded that an important function of insulin consists in its diminishing or inhibiting the overactive gluconeogenesis which is the cause for the excessive sugar production by the liver. Linked closely with this process is that of glycogen formation in the liver (glucogenesis) for

which the presence of insulin is also essential, although available evidence indicates that glycogen formation in the muscles can proceed in the absence of insulin

—J J R M

Progress in the Treatment of Pernicious Anemia

EVERY clinician realizes how unsatisfactory has been the treatment of pernicious anemia. Many different drugs and methods of treatment have been suggested. Only hydrochloric acid in large doses, arsenic and transfusion have stood the test of time as therapeutic measures of proved value. These, however, have only aided in initiating or prolonging the remissions. Nothing has materially altered the inevitably fatal course of the disease.

Most observers agree that true pernicious anemia is of intestinal origin. Numerous workers, beginning with Heiter, have thought that the type of intestinal flora present may be the determining factor in the production of poisons with an affinity for the hematopoietic and nervous systems. The proof offered for this view is not convincing. The same may be said for the theory that certain yeasts may be the causative agent.

Clinicians have emphasized that patients suffering from pernicious anemia have often taken for a long time an incomplete diet. Bohan¹ has especially called attention to the fact that there is commonly a protein deficiency over a long period. Other patients may give a history of taking excess fats. It is also possible that toxic bodies may be formed as the result of altered digestion consequent upon the deficiency of hydrochloric acid in the stomach, a constant finding in the disease.

Koessler, Mauer and Laughlin² think that changes in the viability and permeability of the intestinal wall may be important factors. Certain products of normal digestion are toxic if introduced into the blood stream. Normally the intestinal wall is an effectual barrier to the entrance of poisons into the blood. McGarrison has pointed out the very striking changes which take place in the intestinal wall as the result of vitamin deficiency. Koessler, Mauer and Laughlin produced a marked anemia in the rat by feeding a vitamin deficient diet, which they think is due to altered permeability of the intestinal wall. They state that suggestive good results have been obtained in patients with pernicious anemia by feeding a vitamin rich diet.

Certainly the most promising results so far obtained from any method of treatment of pernicious anemia are those reported recently by Minot and Murphy.³ They have treated a large series of patients with a full protein diet including at least a half pound of liver a day. Fats have been much restricted, and fruits and vegetables supplied in abundance, but liver is seemingly the most important item in the diet. The use of liver is thoroughly justified from experimental work, since Whipple and Robscheit Robbins⁴ found that it is especially valuable in hastening blood regeneration in dogs with experimental anemia.

It is as yet not clear whether the favorable action is due to the fact that liver is a complete protein or contains some specific antihemolytic factor.

Conclusions must certainly be cautiously drawn since pernicious anemia often runs a bizarre course with frequent spontaneous remissions. Clinicians elsewhere are, however, experiencing equally as good results as those reported by Minot and Murphy by the proper use of the diet suggested by the Boston investigators. There is uniformly a rise in the reticulocytes and a fall in the icterus index indicating there is more active regeneration and less active destruction of red cells with the liver diet.

The introduction of this diet by Minot and Murphy marks a distinct advance in the treatment of pernicious anemia.

REFERENCES

- ¹Bohan P T Personal Communication
²Koesler K K, Mauer, Siegfried, and Laughlin Rosemary Jour Am Med Assn 1926, lxxxvii 476
³Minot, S R, and Murphy, W P Jour Am Med Assn 1926 lxxxvii 470
⁴Whipple, G H, and Robschert Robbins F S Am Jour Physiol, 1925 lxxii 395

—R L H



DR. A H SANFORD
Rochester, Minn
President Elect

of Standards, President William G. Epton, Dr. M. T. MacEachern of the American College of Surgeons, and Dr. George W. McCoy, Director of the United States Hygienic Laboratories.

During the six years of the existence of the Society an organization has been built up which has done valued service to the cause of scientific medicine both in private and hospital practice. Closely cooperating with the American College of Surgeons the laboratories in all standardized hospitals have, through the efforts of the American Society of Clinical Pathologists, been placed on a high level of efficiency. The clinical pathologists occupying the directorships of these institutions have been a power and a force in stimulating the attending staffs to the adoption of exact methods in diagnosis and therapy. The laity too has become educated to the important role that the pathologist plays in the teamwork of the hospital and his active collaboration with the clinician.

The date of the meeting has been purposely placed as near to the American Medical Association Convention as possible in order to give our members opportunity to attend the big gathering. They will also be able to take advantage of the reduced transportation rates. By carrying the program over from the week end to Monday, the intervening Sunday can be profitably devoted to committee meetings also the pleasant reunions among comrades in the common cause, the swapping of reminiscences of our early struggles, in the formation of new friendships and pleasant associations. The wives too, and members of families of the Fellows will find in the convention a great opportunity both for sight seeing and social enjoyment. The local committee in Washington is making plans for their entertainment.

The business session promises to be extremely interesting to all the members and they are asked to participate actively therein. It will touch on questions that vitally affect their future. The first topic of discussion will probably center on the report of the State Laboratory Committee. The recent questionnaire sent out to the members has provoked considerable thought and aroused an enthusiastic response. The Committees on Research, Publication, and Registration of Technicians will also present reports which will arouse liberal discussion. It is incumbent on each member to immediately make arrangements to attend the convention. The time spent will be more than compensated for by the stimulation received in listening to the scientific papers and by contact with colleagues in the same planes of activity. He will not only receive information that will be useful to him in his daily practice but will confer the same benefit on his fellow members. It must be remembered that only by combined effort has it been possible to place our specialty on the high plane that it is occupying at present in the field of medicine equal to that of other branches. Your attendance at the Convention will lend encouragement to your fellow workers in the field of clinical pathology.

Judging from the response to our inquiry as to prospective attendance, the Sixth Annual Convention of the American Society of Clinical Pathologists promises to eclipse all previous records.



DR WARD BURDICK
Denver, Colo
Secretary Treasurer



DR J H BLACK
Dallas, Texas
Vice President



DR T B MAGATH
Rochester, Minn
Editor



DR GEORGE IVES
St Louis Mo
Chairman Board of Censors



DR ERNST A VICKERS
San Francisco, Calif
Member of Board of Censors



DR W F THOMSON
Beaumont Texas
Member of Board of Censors



DR PAUL ROTH
Battle Creek Mich
Member of Board of Censors



DR ROBERT A KILDUFFE
Atlantic City N J
Member of Board of Censors
Chairman Service Bureau
Committee



DR H. R. MILLS
Tampa Fla
Member of Board of Censors

American Society of Clinical Pathologists Sixth Annual Convention

WILLARD HOTEL, WASHINGTON, D C

FRIDAY, MAY 13, 9 A M

CALL TO ORDER

SHORT BUSINESS SESSION

Scientific Program

Symposium on Kahn Test

A Study of the Micro Kahn Test in Syphilis By Robert A Kilduffe, M D, Atlantic City, New Jersey

The Microscopic Kahn Reaction By Francis B Johnson, M D, Charleston, South Carolina

Further Studies of the Kolmer and Kahn Tests By C E Roderick, M D, Battle Creek, Michigan

Discussion opened by Dr R L Kahn, Lansing, Michigan

Fatalities Following the Use of Arsphenamine with Report of Autopsy By Ernest Scott, M D, and R A Moore, M D, Columbus, Ohio (Read by title)

Brain Structure Changes After Treatment in General Paralysis By A M P Saunders, M D, Dunning, Illinois

The Use of Injection Methods in Pathology By Ernest Scott, M D, and R A Moore, M D, Columbus, Ohio

FRIDAY, MAY 13, 2 P M

The Blood Picture of Purpura By Nathan Rosenthal, M D, New York City

Anemia as a Factor in the Test of the Rate of Sedimentation of the Erythrocytes By Roger S Hubbard, Clifton Springs, New York (By invitation)

Studies of Sedimentation of Erythrocytes By A H Sanford, M D, I Technic, M D, and H F Hunt, M D, Rochester, Minn

Differential Blood Counts A Comparison of the Accuracy Obtained by Various Methods By Dean N Beacom, M D, Denver, Colo

Ovarian Function Its Influence on the Concentration of Calcium in Blood By Herman Sharlit, M D, and Wm G Lyle, M D, New York City

Purpuric Smallpox, Review of Recent Studies By Kano Ikeda, M D, St Paul, Minnesota

FRIDAY, MAY 13, 8 P M

A Key to the Diagnosis of Neoplasms By Dr Wm Carpenter MacCarty, M D, Rochester, Minnesota

Rapid Methods of Examining Tissue Microscopically Without a Microtome By B F Terry, M D, Rochester, Minnesota

The Present State of Our Knowledge of Gingivitis By Robert A Keilty, M D, Danville, Pennsylvania

The Etiologic and Specific Relationship of Foci of Infection to Certain Organic Lesions A Postmortem Study By A S Giordano, M D, South Bend, Indiana

The Value of Tonsil Cultures in Cases of Focal Infection By Russell Richardson, M D, Philadelphia, Pennsylvania

SATURDAY, MAY 14, 9 A M

Accuracy and Precision in Clinical Pathology By P V Wills, Dr Sc, Newark, New Jersey (by invitation)

New Clinical Methods for Measuring Color and Turbidity as Applied in the Junior Spectrometer By Wm G Exton, M D, Newark, New Jersey

A Turbidimetric Method for Sugar By Anton R Rose, Ph D, Newark, New Jersey (By invitation)



DR. PHILIP HILLKOWITZ
Denver Colo
Chairman Executive Committee



DR WILLIAM CARPENTER MACCARTY
Rochester, Minn
Member of Executive Committee



DR JOHN A KOLMER
Philadelphia Pa
Member of Executive Committee
Chairman, Publication Committee



DR FRANK W HARTMAN
Detroit, Mich
Member of Executive Committee



DR W W COULTER
Houston, Texas
Member of Executive Committee



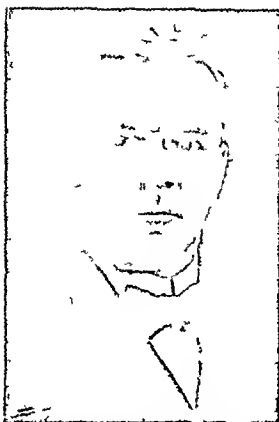
DR HEPMAN SPITZ
Nashville, Tenn
Member of Executive Committee



DR A H SCHAFF
Toledo, Ohio
Chairman, Committee on Exhibits



DR H J COPIE
Denver, Colo
Chairman, Research Committee



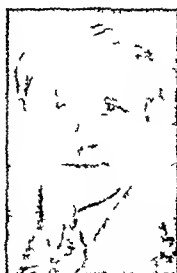
DR. FREDERIC J. SONDERN
New York City
Chairman Public Relations Committee



DR. REUBEN OPPENREICH
New York City
Chairman Program
Committee



DR. GEORGE A. BURGESS
Washington D. C.
Director Bureau of
Standards
Speaker at Annual Banquet



DR. M. T. MACLACHLAN
Chicago Ill.
Associate Director American
College of Surgeons and
Director of Hospital Activities
Speaker at Annual Banquet

- Sugars in Normal Urine By Isadore Greenwald, M D, New York City (By invitation)
 Occurrence of Lipoids in Urine and Their Diagnostic Importance By E L Miloslavich,
 M D, Milwaukee, Wisconsin
 The Comparative Diagnostic Value of the Levinson Test and the Glucose Content of the
 Cerebrospinal Fluid in Tuberculous Meningitis By A S Giordano, M D, South Bend,
 Indiana

SATURDAY, MAY 14, 2 P M

- Pathology of Intestinal Tuberculosis By Alfred Blumberg, M D, Oteen, N C
 The Cultivation of Tubercle Bacilli By H J Corper, M D, and Nao Uyer, Ph D, Denver,
 Colorado
 Some Observations on Basal Metabolism By Leon S Lippincott, M D, Vicksburg, Miss
 Pathological Laboratory Examinations for the Dentist By Charles G Darlington, M D,
 New York City
 Laboratory Examinations Necessary and Unnecessary By George L Schadt, M D, Springfield,
 Massachusetts
 A Modification of the Technique of the Wassermann Test By L H Cornwall, M D, D
 Groszberg, and Blanche C Taylor, New York City

SATURDAY, MAY 14, 7 P M

ANNUAL BANQUET

- The Relation of Clinical Pathology to Preclinical Medicine By President William G Epton,
 Newark, New Jersey
 The Relation and Responsibilities of the Clinical Pathologist to the Hospital Standardization
 Movement By Dr M T MacEachern, American College of Surgeons, Chicago, Ill
 Remarks by Dr Norris Fishbein, Editor, Journal American Medical Association, Chicago, Ill
 Remarks by Dr George K Burgess, Director, Bureau of Standards, Washington, D C
 Remarks by Dr George S McCoy, Director, Hygiene Laboratory, Washington, D C

MONDAY, MAY 15, 1927, 9 12 A M and 2 5 P M

BUSINESS SESSION

- Call to order
 Reading of Minutes
 Unfinished Business
 Reports of Committees
 Committee on Exhibits
 Committee on Public Relations
 Publication Committee
 Program Committee
 Research Committee
 Service Bureau Committee
 State Laboratory Committee
 Committee on Registry of Technicians
 Election of Members
 New Business
 Report of Nominating Committee
 Election of Officers
 Selection of next meeting place
 Adjournment

A cordial invitation has been extended to the members of this Society by the Bureau of
 Standards to visit their Department

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D. Editor in Chief
Medical Arts Building
Richmond Va

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.D.
University of Toronto Toronto

Chemical Pathology

W. C. MACCARTY, M.D.
Mayo Clinic Rochester Minn

Biochemistry

VICTOR C. MYERS, Ph.D.
State University of Iowa Iowa City

Immunology

JOHN A. KOLMER, M.D.
University of Pennsylvania
Philadelphia

Bacteriology

A. PARKER HITCHENS, M.D.
Bureau of Science Manila, P. I.

Internal Medicine

GEORGE R. HEPRMANN, M.D.
Tulane University New Orleans

Pathology

PAUL G. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD B. WEBB, M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR, M.D.
Mayo Clinic Rochester Minn

Experimental Medicine

RUSSELL L. HADEN, M.D.
University of Kansas Rosedale

ROBERT A. KILDUFFE, M.D.
Atlantic City N. J.

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY 3523 25 PINE BLVD ST LOUIS

Entered at the Post Office at St. Louis Mo. as Second Class Matter

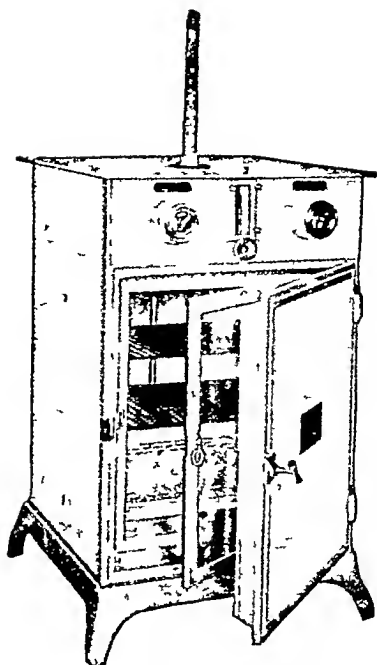
LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment Estimates Cheerfully Furnished Prompt Service

See our advertisements pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA, PA



Freas Incubator No 26

Your Incubator Must be Accurate

Just as with your laboratory worker your incubator must be capable of performing the assigned tasks accurately

Accuracy in an incubator is its ability to maintain the temperature constant continuously

This is why the Freas Incubators are used in the leading laboratories

The Thermo Electric Instrument Co.
1215 South Grove Street, Irvington, N. J.

FREAS

ELECTRIC INCUBATORS

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO, MAY, 1927

No 8

CLINICAL AND EXPERIMENTAL

THE EFFECT OF VENOUS STASIS ON THE PROTEINS OF BLOOD PLASMA AND ON THE RATE OF SEDIMENTATION OF THE RED BLOOD CORPUSCLES*

BY E D PLASS M D, AND M D ROURKE, M S, DETROIT, MICH

IT HAS been well established that venous stasis leads to dehydration of the blood Dautiebaude, Davies, and Meakins¹ have shown that such a procedure causes passage of both electrolytes and water to the tissues, while Grawitz² and Schultz and Wagner³ have demonstrated marked increases in the erythrocyte count, the hemoglobin content, and the specific gravity of the whole blood A rise in total plasma protein percentage during venous stasis has been proved by Kreibich,⁴ Rowe,⁵ Peters Bulger Eisenman, and Lee,⁶ Bohme,⁷ and others Rowe's⁵ studies on the increases of the albumin and globulin fractions of human blood serum in eleven pathologic cases with as many different diagnoses showed that in general the percentage increase of albumin was higher than that of the globulin although three cases show the reverse † Rowe also pointed out that the protein increase is a function of the duration of the stasis and that a stasis as short as one and one half minutes increased the albumin by 5.65 per cent and the globulin by 3.71 per cent Peters, Eisenman, and Bulger⁸ in 1925 reported two experiments on venous stasis where they studied simultaneous changes in total plasma protein percentage and plasma volume percentage and found the plasma protein increase of the same order of magnitude as the plasma volume decrease They explain their results on the theory of simple plasma concentration with no transfer of protein to or from the tissues They disregard however the very marked differences between the percentage increases of the albumin and globulin fractions pointed out by Rowe⁵ and amounting to from 27 to 58 per

From the Obstetrical Department of the Henry Ford Hospital

†Rowe used Robertson's refractometric method

Received for publication, January 7 19

The nature of the physicochemical properties of the superficies of the micellae of any colloidal system is dependent upon the properties of the medium in which the particles are suspended. In turn, the stability of a colloidal system is dependent upon the properties of the superficies. We should, therefore, look for changes in the suspension medium—the plasma—which can affect the physicochemical properties of the superficies of the

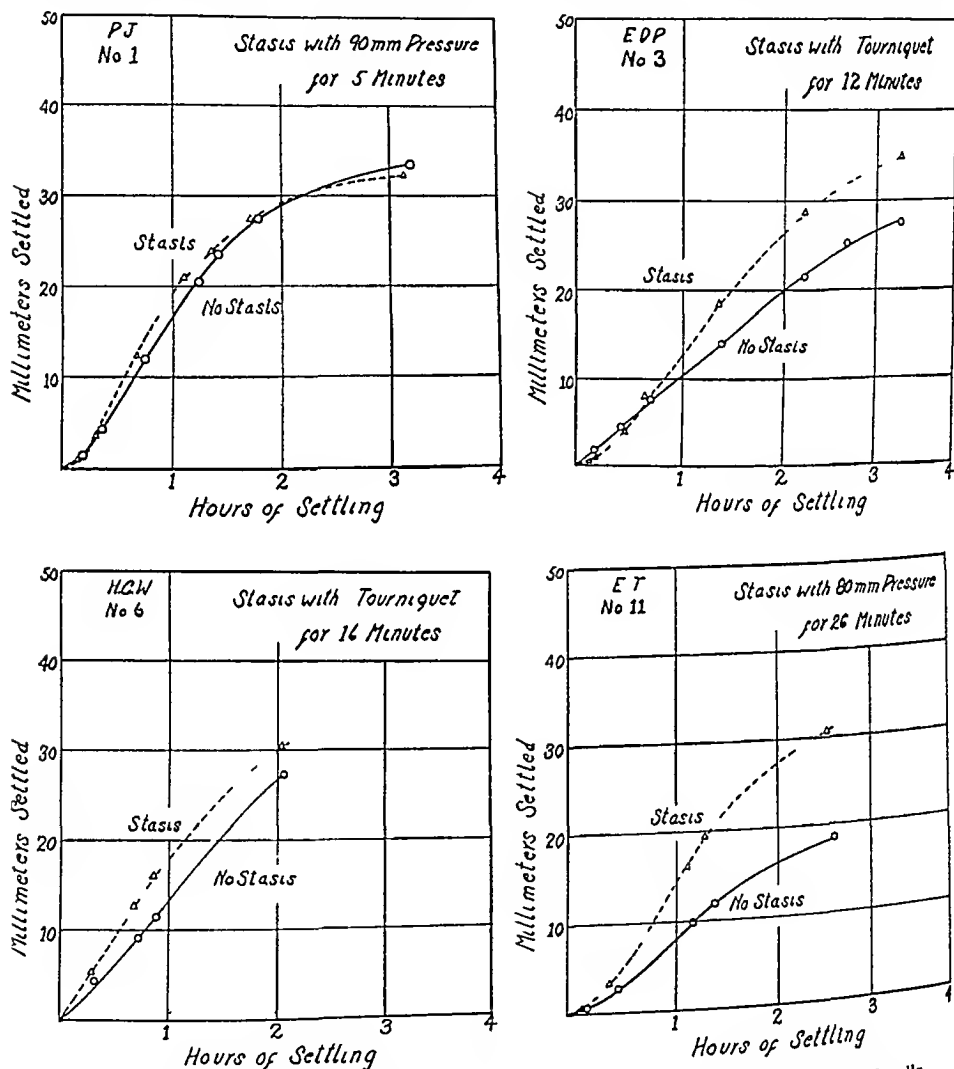


Fig 1—The effect of venous stasis upon the sedimentation rate of the red blood cells.

micellae—the red blood corpuscles—and can alter the stability of the blood as a colloidal system

The P_H of the plasma has been reported by Peters⁶ to be appreciably lowered by venous stasis, but the effect of different hydrogen-ion concentrations on the sedimentation rate of the red blood corpuscles has not yet been studied. Plasma chlorides, as reported by Peters⁷ and confirmed by our experiments, are somewhat lowered. Fahraeus¹⁸ has shown that the addition of sodium chloride decreases the rate of sedimentation. The magnitude of

the decrease of sodium chloride, which is produced by stasis, is, however, very much less than that by which Fahraeus produced his results, and it can hardly be imagined that it could effect changes of the magnitude we have demonstrated. Plasma oxygen and carbon dioxide tensions are also changed by stasis, but the effect of these changes on the sedimentation rate has not yet been studied carefully. Increased corpuscle number has been shown to produce a decreased rate of settling the opposite of the effect we noted from stasis.

Fahraeus¹⁸ and many others have pointed out the important role of the plasma proteins in determining the rate of settling of the red blood cells but there is considerable difference of opinion as to which protein is concerned. Fahraeus feels that an increased sedimentation rate is usually explained by a globulin increase, while Linzenmeier¹⁹ attributes the variations to what he calls the "Seukungsbeschleunigende Substanz" and which he identifies more or less positively as fibrin. Musa,¹⁹ Westergren,²⁰ Gram,²¹ Starlinger,²² and many others attribute an increased rate of settling in certain conditions very definitely to increased fibrin, and we prefer to assume this position, not losing sight of the fact that some of the other factors which have been mentioned may have more or less influence.

SUMMARY AND CONCLUSIONS

Prolonged venous stasis leads to blood dehydration and to an increase of plasma proteins. The fibrin, globulin and albumin are each increased and to a greater extent than can possibly be accounted for by concentration of the plasma alone. The increase in the separate proteins are not equal to each other, nor is any one consistently higher or lower than the others.

We believe that certain catabolic products formed in the tissues by reason of the anoxemia imposed by the venous stasis have acted upon the cell 'membranes' of both the capillaries and the tissue cells, increasing their permeability to protein.

It is suggested that the lymph fluid has increased in protein content by diffusion of protein from the cytoplasm of the tissues which it surrounds and that this lymph fluid, having become higher in protein than normal plasma, subsequently loses protein to the plasma, thereby increasing the total plasma proteins.

The marked increase in fibrin during venous stasis points to reserve fibrin in the tissues.

The sedimentation rate is increased by prolonged venous stasis and it is suggested that the increase is probably due chiefly to increased fibrin.

Care should be exercised in the use of the tourniquet or other constriction during the taking of blood upon which the sedimentation rate or plasma proteins are to be determined.

REFERENCES

- ¹Dautrebande L., Davies H. W. and Meakins J. The Influence of Circulatory Changes on the Gaseous Exchange of the Blood. III. An Experimental Study of Circulatory Stasis, Heart 1923 v. 133 152.
- ²Grawitz, E. Klinisch experimentelle Blutuntersuchungen Ztschr. f. klin. Med., 1892, xxi 409 474.
- Idem. Klinische Pathologie des Blutes Leipzig 1911, Georg Thieme.

element also have a part. Application of the simple tourniquet usually did not produce so great a change as the pressure controlled by the arm band of the sphygmomanometer. The pressure exerted by the former undoubtedly varies considerably since there is no true method of control within wide limits.

In no case of the eleven reported is the plasma volume percentage decrease even substantially equal to the total protein increase or equal to the fibrin, globulin, or albumin increase when considered alone. The change in plasma volume is due almost entirely to loss of fluid to the tissues as is shown in Experiment No. 9, where the cell volume increase was found to be 16.9 per cent and the cell count increase 18.2 per cent. The increase of total protein or of any particular protein cannot, therefore, be ascribed to a simple loss of fluid to the tissues. That no appreciable increase of the protein content of the plasma could be effected by synthesis of the amino acids and peptid nitrogen is shown by the constancy of the nonprotein nitrogen figures during stasis reported by Rowe and confirmed by us in Cases No. 9 and No. 10, where the nonprotein nitrogen rose from 39.2 to 50.0 mgm. per 100 cc., and from 30.8 to 36.1 mgm. per 100 cc. respectively. This apparent rise can be explained by the increased cell numbers, cells being higher in nonprotein nitrogen than the plasma. The initial high value in the first instance may be due to the fact that the specimens were taken about one hour after breakfast. That the increase in plasma protein cannot be accounted for by a decrease of corpuscle protein is shown by experiments on Cases No. 9 and No. 10, in which the corpuscle protein changed only from 38.5 to 38.6 grams per 100 cc., and from 36.3 to 34.7 grams per 100 cc., respectively, due to stasis. The whole blood protein was determined by the Kjeldahl method and the corpuscle protein calculated from these results together with the plasma protein and the cell volume figures.

Staling¹¹ has shown that normally the lymph in the extremities contains only from 2 to 4 per cent protein, the lymph in the intestines from 4 to 6 per cent protein, and that in the liver from 6 to 8 per cent protein. From these facts and a consideration of Traube's theory of membrane permeability we might conclude that the sieve structure of the capillary walls of the liver is coarser than the structure in the extremities and allows the large hydrated solute molecules, the proteins, to pass more easily through the interstices. In certain pathologic conditions, wound shock,¹² hemorrhagic shock, histamine shock,¹³ and others, it has been shown conclusively that the capillary walls in the extremities have become much more permeable to protein and that the formation of lymph has increased, thus dehydrating the blood. Heidenhain¹⁴ has shown that peptone is a powerful lymphagogue and that the lymph formed may be richer in protein than the blood plasma.

The tissues of the arm during stasis suffer from acute anoxemia. Under this condition, fixed acid products are formed within the tissue cells as soon as the process of oxidation is handicapped (Koehler, Brunquist, and Loevenhart¹⁵). The carbon-dioxide content must increase continually both in the tissues and the blood in the absence of a means for its removal or of a sufficient supply of reserve alkali. Consequently, there has been induced a tissue and blood

acidosis⁶ That this critical state of anoxemia and acidosis in the tissues has probably led to the formation of products which affect the physical chemical properties of the membranes, increasing their permeability, and which bring about tissue hydration, is shown not only by the sudden local change of plasma protein in our experiments and those of Rowe et al, but also by a very conclusive experiment of Mann¹⁶ This latter observer produced venous stasis in the four extremities of a dog for an extended period and observed that the animal went into shock when the tourniquets were removed allowing the products which had accumulated in the extremities to circulate throughout the system

As a possible explanation for the increased protein content of the plasma we suggest that products resulting from the anoxemia have altered the permeability of the cell membranes, allowing protein from the cytoplasm, admittedly high in protein to diffuse to the lymph and thence to the blood through the capillary walls, to aid in preserving the osmotic relationships which have been disturbed by the changed physical and chemical conditions

The prompt and large increase in the plasma fibrin during venous stasis is a very interesting observation The fibrin increase is local to the arm from which the blood flow is blocked and cannot therefore, be attributed to any specific liver stimulation This observation would seem to demand that there be reserve fibrin in the tissues of the arm as has been suggested by Foster and Whipple¹⁷ and others

SEDIMENTATION RATES

The sedimentation rates of the red blood cells increased during stasis in all cases except No 2 where the total protein rise is smallest and where there appears to be an abnormal suspension stability without stasis Fahraeus¹⁸ has shown that the rate of sedimentation is influenced by the corpuscle number, all other factors being constant and that, the higher the corpuscle number, the slower the rate of sedimentation He also showed that the sinking velocity is about doubled by a reduction of the corpuscle number from 5 000,000 to 4 000,000 per cubic millimeter Stasis increases the corpuscle number, which effect would tend to decrease the rate of sedimentation barring other changes in the blood Therefore, the difference between any two curves representing the sedimentation rates with and without stasis would be even greater were the corpuscle number the same in the two samples

The same general type of curve is followed in most cases The rise during the first few minutes is less sharp, as is characteristic of the period of primary agglutination, and is followed first by the period of sedimentation which is not impeded by packing and then by the portion showing the damping of the rise due to packing of the corpuscles In practically every case, the curve representing the stasis rate crosses or tends to cross the curve representing the normal rate as they both approach their limits of sedimentation. Since the hematocrit reading is higher for the blood collected during stasis, the limit of plasma height is lower and therefore the curve representing the stasis rate must cross the normal

We are not able to say definitely which of the variable factors brings about an increase in the rate of sedimentation during stasis

The nature of the physicochemical properties of the superficies of the micellae of any colloidal system is dependent upon the properties of the medium in which the particles are suspended. In turn, the stability of a colloidal system is dependent upon the properties of the superficies. We should, therefore, look for changes in the suspension medium—the plasma—which can affect the physicochemical properties of the superficies of the

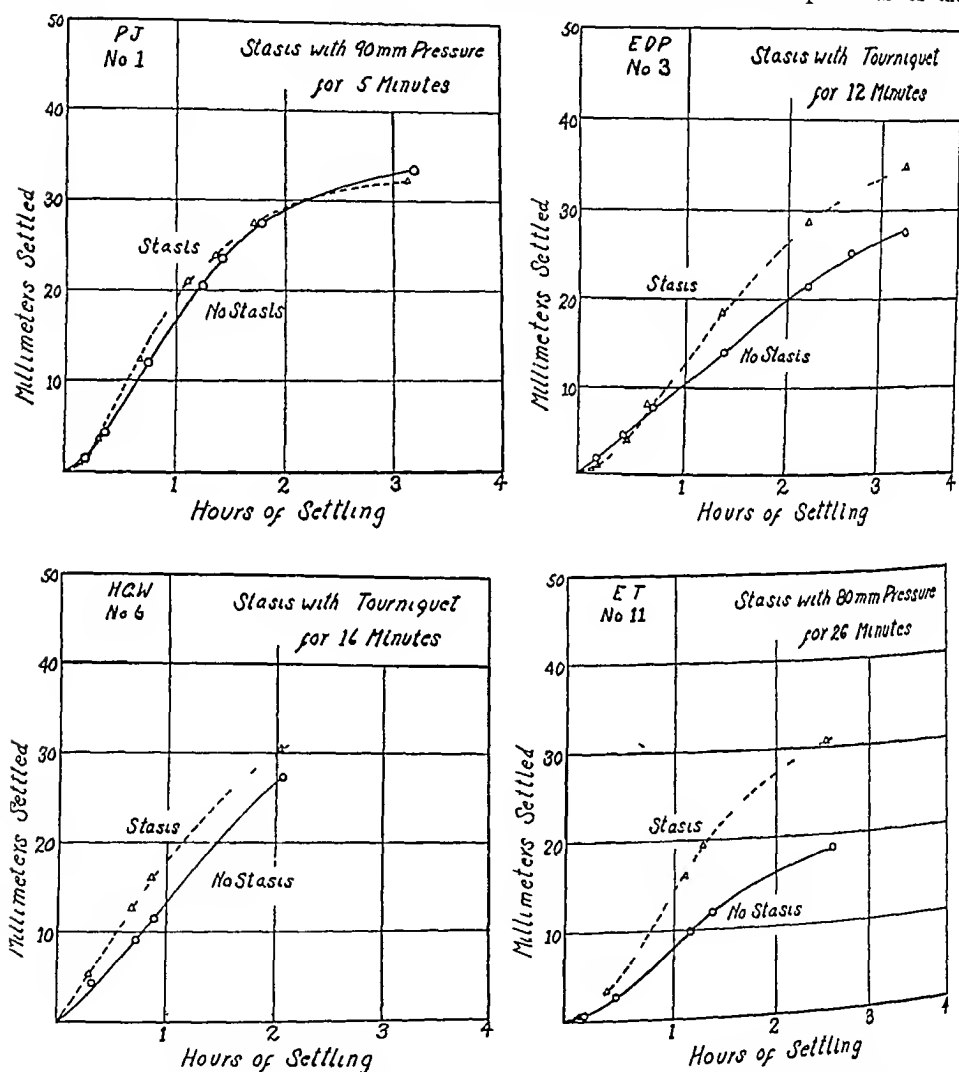


Fig 1—The effect of venous stasis upon the sedimentation rate of the red blood cells.

micellae—the red blood corpuscles—and can alter the stability of the blood as a colloidal system

The P_H of the plasma has been reported by Peters⁶ to be appreciably lowered by venous stasis, but the effect of different hydrogen-ion concentrations on the sedimentation rate of the red blood corpuscles has not yet been studied. Plasma chlorides, as reported by Peters⁷ and confirmed by our experiments, are somewhat lowered. Fahraeus¹⁸ has shown that the addition of sodium chloride decreases the rate of sedimentation. The magnitude of

the decrease of sodium chloride, which is produced by stasis, is, however, very much less than that by which Fahraeus produced his results, and it can hardly be imagined that it could effect changes of the magnitude we have demonstrated. Plasma oxygen and carbon dioxide tensions are also changed by stasis, but the effect of these changes on the sedimentation rate has not yet been studied carefully. Increased corpuscle number has been shown to produce a decreased rate of settling the opposite of the effect we noted from stasis.

Fahraeus¹⁸ and many others have pointed out the important role of the plasma proteins in determining the rate of settling of the red blood cells but there is considerable difference of opinion as to which protein is concerned. Fahraeus feels that an increased sedimentation rate is usually explained by a globulin increase while Linzenmeier¹⁹ attributes the variations to what he calls the "Senkungsbeschleunigende Substanz" and which he identifies more or less positively as fibrin. Musa,¹⁹ Westergren,²⁰ Gram,²¹ Starlinger²² and many others attribute an increased rate of settling in certain conditions very definitely to increased fibrin, and we prefer to assume this position, not losing sight of the fact that some of the other factors which have been mentioned may have more or less influence.

SUMMARY AND CONCLUSIONS

Prolonged venous stasis leads to blood dehydration and to an increase of plasma proteins. The fibrin, globulin, and albumin are each increased and to a greater extent than can possibly be accounted for by concentration of the plasma alone. The increase in the separate proteins are not equal to each other, nor is any one consistently higher or lower than the others.

We believe that certain catabolic products formed in the tissues by reason of the anoxemia imposed by the venous stasis have acted upon the cell "membranes" of both the capillaries and the tissue cells increasing their permeability to protein.

It is suggested that the lymph fluid has increased in protein content by diffusion of protein from the cytoplasm of the tissues which it surrounds and that this lymph fluid having become higher in protein than normal plasma subsequently loses protein to the plasma thereby increasing the total plasma proteins.

The marked increase in fibrin during venous stasis points to reserve fibrin in the tissues.

The sedimentation rate is increased by prolonged venous stasis and it is suggested that the increase is probably due chiefly to increased fibrin.

Care should be exercised in the use of the tourniquet or other constriction during the taking of blood upon which the sedimentation rate or plasma proteins are to be determined.

REFERENCES

- ¹Dautrebande L, Davies H W and Meakins J. The Influence of Circulatory Changes on the Gaseous Exchange of the Blood. III. An Experimental Study of Circulatory Stasis. *Heart* 1923 v 133 152.
- ²Grawitz E. Klinisch experimentelle Blutuntersuchungen, *Ztschr f klin Med*, 1892, xxi 409-474.
- Idem. *Klinische Pathologie des Blutes* Leipz 1911, Georg Thieme.

- ³Schultz, W, and Wagner, G Ueber den Flüssigkeitsaustausch zwischen Blut und Geweben unter den Einwirkung von thermischen und anderen Einflüssen, *Fol Serol*, 1909, iii, 387 420
- ⁴Kreibich, K Ueber die refraktometrischen Werte des Blutserums, *Fol Haematol*, 1907, iv, 795 798
- ⁵Rowe, A H The Effect of Venous Stasis on the Proteins of Human Blood Serum, *Jour. LAB AND CLIN MED*, 1915 1916, i, 485 489
- ⁶Peters, J P, Bulger, H A, Eisenman, A J, and Lee, C Total Acid Base Equilibrium of Plasma in Health and Disease IV The Effects of Stasis, Exercise, Hyperpnea, and Anoxemia, and the Causes of Tetany, *Jour Biol Chem*, 1926, lxxvii, 175 217
- ⁷Bohme, A Ueber die Schwankungen der Serumkonzentration beim gesunden Menschen, *Deutsche Arch f klin Med*, 1911, cxi, 522 562
- ⁸Peters, J P, Eisenman, A J, and Bulger, H A The Plasma Proteins in Relation to Blood Hydration I In Normal Individuals and in Miscellaneous Conditions, *Jour Clin Inv*, 1924 1925, i, 435 450
- ⁹Wu, H A New Colorimetric Method for the Determination of Plasma Proteins, *Jour Biol Chem*, 1922, li, 33 39
- ¹⁰Linzenmeier, G Untersuchungen uber die Senkungsgeschwindigkeit der roten Blutkörperchen, *Arch f Gynak*, 1920, cxvi, 608 632
- ¹¹Starling, E H The Arris and Gale Lectures on the Physiologic Factors Involved in the Causation of Dropsy, *Lancet*, 1896, i, 1267 1270, and 1331 1334
- ¹²Bayliss, W M Intravenous Injection in Wound Shock, *Brit Med Jour*, 1918, i, 553 556
- ¹³Bodansky, M Plasma Proteins in Experimental Anhydremia, *Jour Biol Chem*, 1926, lxxvii, Proc Am Soc Biol Chem, p xxxviii
- ¹⁴Heidenham, R Versuche und Fragen zur Lehre von der Lymphbildung, *Arch f d. ges. Physiol*, 1891, xli, 209 301
- ¹⁵Koehler, A E, Brunquist, E H, and Loewenhardt, A S The Production of Acidosis by Anoxemia, *Jour Biol Chem*, 1925, lxxiv, 313 323
- ¹⁶Mann, F C Studies on Experimental Surgical Shock, *Am Jour Physiol*, 1918, xlvii, 231 250
- ¹⁷Foster, D P, and Whipple, G H Blood Fibrin Studies IV Fibrin Values Influenced by Cell Injury, Inflammation, Intoxication, Liver Injury and the Eck Fistula, *Am Jour Physiol*, 1921 1922, lvi, 407 431
- ¹⁸Fahraeus, R The Suspension Stability of the Blood, *Acta Med Scandm*, 1921, lv, 3 22
- ¹⁹Musa, G Ueber die Senkungsreaktion der roten Blutkörperchen und ihre Ursachen, *Klin. Wchnschr*, 1923, ii, 1591 1592
- ²⁰Westergren, A Studies on the Suspension Stability of the Blood in Pulmonary Tuberculosis, *Acta Med Scandm*, 1920 1921, liv, 247 282
- ²¹Gram, H C On the Causes of the Variations in the Sedimentation of the Corpuscles and the Formation of the Crusta Philagistica on the Blood, *Arch Int Med*, 1921, xxviii, 312 330
- ²²Starlinger, F Zum Funktionsnachweis und Funktionsprüfung der Schilddrüse, *Wiener klin. Wchnschr*, 1922, xxxv, 473

THE PATHOGENICITY OF THE SMALL RACES OF THE "AMEBA OF DYSENTERY" *

BY RAWSON J. PICKARD, M.D., SAN DIEGO, CALIFORNIA

CONJOINT life is a test of strength between its participants. The relationship is seldom a true symbiosis, of benefit to both; practically always one party is the giver, and even when he gives from excess he must always be on guard lest his partner, flourishing, does not take from his need. Each organism strives for the full development of its potentialities. A change in environment changes their relative strength so that from a benign commensalism in which the lesser lives upon the waste of the greater they may pass into a parasitism causing the disease or death of one of the participants. Thus a parasite ignored in its weakness may attack from strength, or from numbers, when conditions favor it. So as with any form of life, the balance is not stable, changing surroundings release or inhibit inherent capacities of expression which bring about physiologic changes often accompanied by changes in morphology. With two different species in some form of conjoint life the balance in their relationship is the more easily disturbed in that the advantages to be seized by one or the other are multiplied by the differences in capacity of expression between different kinds of organisms. And peace is never declared in nature.

In discussing the relationship between man and the ameba or other intestinal protozoa, we properly use in our terminology such terms as variable virulence, immunity, and so on from the partisan point of view of mankind, but in studying these organisms we avoid prejudicing a true estimate of the status of any parasite by the careless use of words which are applicable in an opposite sense from the parasitic standpoint.

The difficulties in the study of human intestinal protozoa are great. The careful study of parasitic protozoa in man is only a few years old. Few of these protozoa have been cultivated at all; the ameba of dysentery only recently and then in association with bacteria. The protozoa are not only more difficult to study than the bacteria but they are also more difficult to recognize. Their more complex forms are often degenerated living or dead when discovered and sufficiently resemble other cells of various kinds to require considerably more specialization to recognize than is required for a bacterial diagnosis. Clinically the difficulties are even greater. "We have been highly trained in thinking from the bacterial point of view since the days of Pasteur and poorly trained in thinking from the point of view of protozoan infection," says Kofoid.¹ We are carrying over into the domain of parasitic protozoology ideas that belong to bacteriology. With bacterial infection are the classic *rubor, dolor, tumor, calor*, and characteristic changes in the blood. Haugh

*Received for publication, January 18, 1924.

wout,² in an early paper on the pathogenicity of the flagellates, spoke of the remarkable powers of adaptation shown by both the free living and the protozoa* and asks, "Does it not seem that we are dealing with affinities of a different nature, chemical reactions governing the diseases of a characteristically nonfebrile character unaccompanied by phenomena of immunity? These phenomena need not be restricted necessarily to the metabolic chemistry of the parasite. They might be due to chemical changes originating in the cells and body fluid of the host." Haughwout was the first to perceive that the protozoan diseases are in nature from the bacterial diseases, and require special methods of treatment, methods perhaps only in the devising at present.

Several of the species of human entamebae are regarded as remaining as harmless commensals, the flagellates sometimes seem to be like the ameba of dysentery, now found to be a common inhabitant of the intestine, while amebic dysentery is rare—in the temperate zone—its status again brought in question. Yet with any parasite, however, the association may appear as deduced from the crude tests we apply in our laboratories, there exists a complex counterreaction between the host and the parasite, the complexity increasing with the difference of the organs of the associates. At one extreme the protozoan is quite and evidently harming its human host, at the other extreme the protozoan is not only well enough tolerated that no signs of damage can be discerned on the basis of encystment, might be said to be on the defensive. The primary dismissal of species which are probably not pathogenic, as is, however, is not a "scientific" attitude. While it would doubtless be not to admire the hesitation of the zoologists to condemn a human parasite in the absence of complete proof of its noxiousness, they judging for matters as they properly judge in other biologic questions, yet it is remembered in favor of physicians, when they look with mistrust on the sites, that medicine, like war, is primarily an art that utilizes science with a necessary admixture still of wit (again like war)—and the clinician in the presence of a sufferer, feels that the individual cannot wait for a tide of absolute conviction to arise in the scientific sea, for the doctor knows his ill. The clinician may well let an empiricism be his guide until one be found, and use on insight like that which led Goethe to a divine truth, the substance of evolution. A working hypothesis based on the facts is of more use to the sick man than to wait for missing links which unite the facts and theory into the force of a law. It is necessary for the physician to remember that he is working on a theoretic basis and he be ready to discard it for one better substantiated, nor must he himself think that one case recovered or cured is proof for the moment's favorable idea.

We are at present sufficiently informed to say that parasite, and commensal, is the term which properly describes the simpler organisms

*Some interesting instances of protozoan adaptability are given in a paper by Dr. J. H. Henshaw, "The development of pathogenicity in the International Conference on Health in Tropical America 1924 United Fruit Co. Boston."

live in more complex forms. Their secret and excreted are certainly unfitted for absorption and use by the higher organism since the protozoan, dependent on the host for its food must utilize this food according to its own metabolic processes which are unquestionably different from those of the host. Therefore during the course of an infection there must be produced products which if absorbed by the host will affect his welfare in proportion to the delicacy and sensitiveness of the retention of his body, largely a function of his body complexity and especially that of the neuropsychic apparatus. These are in addition to any products that might arise from a disease affecting the parasite itself, such as immune bodies against the host, or from the death and disintegration of the parasite whether the parasite remain in the lumen of the bowel or penetrate the tissue and cause direct trauma and periteneal absorption. Any such toxic products although not resorbed would be a menace so that the harmlessness of these organisms must be proved rather than their pathogenicity defended. The most complex organism is man. With his highly developed nervous system man is psychically sensitive to the slightest influence and so sensitive an organism has no 'harmless commensal'. All parasites, in one way or another affect him although it be but slightly. Man's adaptability, marvellously developed may cover the losses or damages for a time or even for a lifetime but in any case there will be individuals who testify to the dangers latent in a parasitism by abnormality of physiology, or by nervous or psychic reaction. Even our inevitable and universal 'commensal,' *B. coli* has had some evidence brought against it as shortening our lives: brief experimental periods have exploded the theory that the intestinal bacteria were necessary to life, and all know that *B. coli* occasionally develops its potentialities to the point of causing acute illness.

I recall two instances of the absorption of products definite at least in the effects produced caused by parasites as far apart as *Hymenolepis nana* and *Entamoeba histolytica* and trichomonas in a double infection. The patient with the dwarf tapeworm, seen in Panama in 1912 had constant fatigue, with attacks of overpowering somnolence which nearly cost him his position. During one course of four weeks' treatment he passed 72 000 tapeworms (there was of course antereinfestation period about seven days), and he was never entirely freed from the infection during the three years I was able to follow his case, but was symptomatically relieved and kept in good health by occasional dosage with aspidium. The other patient Case 17 reported previously,³ had lethargy with attacks of somnolence during which he would fall asleep while walking riding horseback etc. His attacks disappeared with the amebae. Many symptoms in cases of amebiasis can be more plausibly explained and the pathogenesis is more comprehensible if we consider that the metastatic growth of the amebae is less common than the absorption of the products of their growth resulting in affecting with toxins tissues situated at a distance from the site of the amebic proliferation such localization in any special tissue being due either to a specific tropism of the tissue cells for the absorbed molecule, or to a greater susceptibility of the tissue due to strain (joints), or structural delicacy (nerve). Barrow⁴ says there is a toxemia or systemic poisoning from amebiasis manifested by a disturbance in the skin, joints,

blood-forming organs, endocrine function, metabolism, nervous system, or by psychic balance, a poisoning as definite in its clinical aspects as that of any mineral poison. Toxicity and actual tissue invasion are not always clearly distinguishable with present methods. A case illustrative of the theory of a toxic absorption from amebiasis is that of a dentist, Dr. L., who had attacks of sciatica of increasing severity for over a year, and had had all the laboratory tests, clinical, radiologic and protein tests that are available, all negative. The fecal examination was reserved to the last, and numerous E. histolytica found, containing blood cells. He had never had dysentery, had lived in Kansas before coming to San Diego, and had not had tropical contact. The nerve pain disappeared, lagging a few weeks after treatment of the amebae with stovarsol, but returned in three months, at which time the amebae were found numerous again, no treatment having been given in the interval, purposely. He is now well of his sciatica, and the amebae have not increased to the level at which they can be found in the stool, due to regular short courses of stovarsol. In this case the possibility of an occupational (position) neuritis was ruled out, first, as the pain was right sided only, the strain, however, was probably the determining factor in making this nerve the situs minoris resistentiae for a nerve poison absorbed from the activities of the amebae. It is hardly likely that in cases like this, or like those cases of neuritis reported by Mills,⁵ occurring "during the course of a nondysenteric amebiasis," that there is an actual invasion of the amebae in the nerve or in the eye, although by the weight of evidence there can be no doubt that the symptoms are due to the amebiasis. Of course amebae have been found in many parts of the body, carried by the blood stream, as can be found in the list of references given by Mills in his paper and by Kofoid and associates in their paper on "Systemic Infections by Entameba Dysenteriae."⁶ The finding of amebae in certain cases of arthritis by Kofoid and Swezy⁷ has not been confirmed so far, nor has their demonstration of amebae in Hodgkin's disease, due probably to the difficulties and time required for thorough search. With the discovery of better methods of culture the question of the actual presence of the amebae in these and other lesions may be settled, one recalls the difficulty of finding the streptococci in joint lesions before a special technic was developed, and the long time that these arthritic diseases were attributed to a diathesis—for which, one wonders, the present day fashionable synonym may be "toxicity."

In bacterial feeding there is necessarily an excretion of the digestive enzymes in order to liquefy the food outside the bacterial body so that it can be absorbed. These enzymes and the excreta of bacterial catabolism account for nearly all the action of bacteria upon the human body. A pathogenic bacterium producing a large amount of enzyme to digest a resistant food might be said to have an increased virulence. Most of the amebae and flagellates in the human intestine live free in the lumen, and obtain their food, consisting chiefly of bacteria and yeast cells, occasionally cellular detritus or other protozoa, by engulfing it entire into a food vacuole from which it is absorbed. Except for the dejecta of life processes there is no product necessarily excreted by the intestinal protozoa either to obtain their food, which they are able to capture by movement and do not appear to paralyze, or to

predigest it. There is the notable exception of *Entameba histolytica* which is entirely or almost entirely a tissue feeder* eroding or penetrating the tissues of its host by means of a cytolytic solvent so subtle that the phagocytic cells of the host are not alarmed. Rarely does it ingest blood cells except when present in the dysentery of which it is the cause. But our ignorance of the metabolic processes of the intestinal protozoa amounts to almost totality, due to our inability to grow them in pure culture. Even the histolytic ferment of the ameba of dysentery is known only by its effect, visible enough even in stained tissue sections in the clear liquid zone surrounding the amebae that have invaded the tissues. While we are awaiting more exact information it is best, as Kofoid advises, to record all parasites found in each case, carefully to distinguish the species, to study the history and symptomatology of the patient in the greatest detail, and in general proceed in a manner calculated to increase our knowledge rather than to pass without notation the infections that seem harmless in present light. We are fortunate to have the enthusiasm and accurate observation of a protozoologist like Kofoid working on these problems in medical territory with resources that promise solutions for many of them.

Omitting the amebae reported from a few isolated cases six species have been described occurring as parasites in the intestines of man. There is considerable confusion in the nomenclature so that the older papers are unavailable for the ordinary student on account of the numerous synonyms, ten to twenty for each species and even today the authorities have not agreed upon the correct names for the different amebae. The ameba of dysentery is *Entameba histolytica* for Dobell, *Endameba histolytica* for Hegner and Taghaffer⁹ *Entameba dysenteriae* for Brumpt and for Kofoid. *Endolimax phagocytoides* (B), *Endolimax nana* (H and T, D and O C) is *Entameba nana* for Kofoid. *Iodameba butschli* (D), *Iodameba williamsi* (H and T), *Endolimax williamsi* (K), is *Pseudolimax wenyoni* for Brumpt. Since the writings of these authors are on the ready reference shelf of laboratories doing fecal diagnosis, it is unfortunate that there is not the unanimity in names for these amebae that there is with *Entameba coli*. *Councilmaniana laffleuri* (Kofoid), not generally recognized yet, is listed by Brumpt¹⁰ among the synonyms of *E. coli*, which he speaks of as being "totally inoffensive" while giving in a foot note reference to the observations of Riff, of Strasbourg who found it in cysts of the appendix and of the tubes. In some instances *E. coli* may have been confused with the pathogenic *Councilmaniana laffleuri*, in others it has been sufficiently identified as the cause of an enteritis. Crowell¹¹ says that the position of *E. coli* is not certain some do not concede its lack of pathogenicity, finding cases of dysentery in which only *E. coli* is found. Brown¹² reports several cases in which *E. coli* was without doubt pathogenic, and also reports three cases in which *Endolimax nana* (*Entameba nana*) seemed responsible for a severe diarrhea. This ameba has hitherto been considered harmless. *Dientameba fragilis* and *Iodameba butschli* have never been found in suspicious circumstances.

Thus the only regular offender is *Entameba histolytica*, and until the extensive stool surveys were made during the war, its role was seldom ques-

tioned. Then, finding this ameba in large numbers of apparently healthy carriers cast doubt on its pathogenic rôle, and brought about a change of opinion, more among the zoologists than among the clinicians. Hegner⁹ says that in spite of *E. histolytica* living only as a parasite on the tissues, not more than 10 per cent of the people infected show any marked clinical symptoms. The "size races" seemed to offer an explanation, at least in part. Brumpt¹⁰ noted that the "minuta" forms had never been seen in the liver, that they were not blood-feeding, and were the type found in (so called) healthy carriers and convalescents. Kofoid¹¹ states that while we have no information as to the meaning of the size races, that when the clinical histories indicate a long standing infection, the small race, with cysts from 4 to 6 mu in diameter, are found, and are suggestive of a possible modification in size as a result of long contact with the host. "Cases of human infection observed over months, or at intervals of years, reveal a constancy of cyst dimensions indicating that changes do not occur quickly." Kessel¹² found distinct size races in rat ameba which remained constant during passage through culture rats over a long period. Dobell⁸ said that the races differing in size differed in no other characteristic, morphologic or physiologic, but he wrote this in 1921. Brumpt, in the 1922 edition of "Parasitologie," describes two cycles in the evolution of the dysenteric ameba, "a normal, nonpathogenic cycle," and the abnormal pathogenic cycle. In the carriers the ameba is always of the small size race, multiplies by scission, and forms the cysts with four nuclei. The abnormal cycle "is brought on by the influence of intercurrent disease, parasitic associations, or following modifications of the intestinal status" or changes in the general health of the host, which cause the ameba to develop tropisms unfavorable to the host. This transformation, exceptional in man (2 to 3 per cent), is the rule in the cat when it is infected by the ingestion of ripe cysts. The small races will cause dysentery in cats, he states, yet no one has explained how the minuta forms become hematophagic and pathogenic. "The hematophagic amebae multiply rapidly by scission and are rapidly eliminated with the quantities of mucus coming from the intestinal irritation which they provoke." The question of size races is much confused. The change in size on feeding cats with cysts of races with small cysts, should be confirmed by the careful measurement of ripe cysts later obtained from the cat, to determine whether the small precystic ameba had not merely grown into the larger, hematophagic, rapidly multiplying form, and did not again produce small cysts when conditions again favored encystment. The same change in size of motile forms can be brought about in culture amebae by a meal of red blood cells. Reports from various workers that they have "seen the small race change to the large during a relapse of dysentery," and vice versa, have not been based on cyst measurements. Again it is not infrequent that the two size races exist simultaneously, a larger ameba with large sized cysts ingesting blood cells and the patient suffering from dysentery, while a small race with small cysts coexists, of uncertain rôle. Such was the case of a patient recently referred by Dr. Alberty for fecal examination. The patient had never lived away from the northern Middle West, and had had dysentery for about a year. In this case we do not know but that this man had long been

a carrier of the small race of *E. histolytica*, and had a secondary infection with the larger race that produced the dysentery the past year

There seems to be a distinct difference in the pathogenicity of the size races. The small races are widely diffused throughout all races of mankind, and family infections are common. Kofoid¹³ says that patients reporting no history of dysentery form by far the greater proportion of their records, but adds that "such testimony could hardly be expected to represent accurately the period of infancy, within which amebiasis may well have been required, and may have been accompanied by an initial attack of dysentery followed by the chronic carrier stage, whose most evident intestinal symptom is constipation." In many examinations of the stools of infants and children with diarrhea and dysentery in California I have never found amebae, although about 15 per cent of adults referred for examination because of intestinal symptoms are carriers of *E. histolytica*. Certainly the small races do not ingest blood cells and in the nondysenteric amebic diseases are the type usually found. In the case of 'arthritis deformans of Ely's second type and of Hodgkin's disease as well as many cases of chronic, low grade ill health,'" Kofoid⁶ finds the size of the cysts generally less than 10 microns, often 7 to 9, sometimes 6 to 8, rarely from 3 to 5 microns. Such small amebae could pass through any capillary.

Until the culture of the ameba is simpler than it is at present, the problem cannot be solved whether the large forms are activated hematotropic ameba of the same race as the small amebae or really a different infectious "In any case this question of races presents a considerable biologic interest for the problem of immunity," says Brumpt.¹⁰ Indeed, if there really are different races, it might be asserted that the infected individual is in a state of anergy, since, in spite of the new infections by cysts from races differing in size, he shows only the race that infected him originally. On the contrary, if the dwarfism or giantism of the cysts is due to the surrounding medium, it is impossible for us to know whether superinfection is possible or not and whether there is a relative immunity in the course of intestinal amebiasis." As above stated, we do find individuals with coexistent different size race infections but have no means of telling whether the one size race has not developed from the other. The possibility of immunity in the rat Kessel thinks is suggested by his experimental facts. Dobell and O'Connor³ say that all the evidence goes to show that whether the infected individual suffers or not from his infection depends rather on his own susceptibility than on the virulence of the parasite. Haughwout² says that it is hard to escape the conclusion that the host on occasion may transform an apparently harmless parasite into one that is pathogenic apart from lowered vitality and resistance.

There is today little doubt that *E. histolytica* is more or less pathogenic to any individual who harbors it. Dobell says *E. histolytica* is a true tissue parasite facultatively pathogenic. "There can be no doubt that the carrier of *E. histolytica* though he display no symptoms always has a more or less eroded or ulcerated gut." "Amebic infections are very persistent probably life long unless eradicated by specific treatment. Consequently all who once become infected with this parasite are likely to suffer from amebic diseases at

some subsequent time. For the average case the risk is probably small." There was lacking until quite recently the thorough search for symptoms in the past or present of such carriers and the proof that, when the number of amebae has risen to the level at which they may be found in a fecal examination, they are numerous enough to cause symptoms. This work has largely been done by Kofoid and his followers.

We have long known that the ameba living in the mucosa of the colon invaded the vessels and found its way to the liver, and occasionally to the lungs, usually by extension, and rarely was carried in the blood to the brain. Later, amebae were found in the urinary tract, testis, fallopian tubes, and spleen, and finally in the bone marrow in certain cases of arthritis, and in the lymph glands in Hodgkin's disease by Kofoid, Boyers and Swezy,⁶ who "connect this ameba with a widely prevalent mild type of invalidism in middle age and thereafter."

Boyers, Kofoid and Swezy¹ state their present concept of chronic amebiasis with *E. histolytica* (vel *E. dysenteriae*) as a definite clinical entity, recognizable as such. They find a marked fatigability, commonly associated with constipation or a constipation broken by evanescent diarrheas, abdominal soreness, digestive disturbances and neurosis of vague nature. "The normal man knows hunger and the desire for evacuation, otherwise his bowel does not obtrude itself on his consciousness. In amebiasis he is not comfortable in the abdominal region, he is "bowel conscious", as a rule he has been previously diagnosed as having chronic inflammation of various organs, and sometimes, unfortunately for the patient and for the reputation of the operator, there is a history of operations without relief, in Barnow's cases,⁴ 28 per cent had had gastrointestinal operations. "Human amebiasis is a definite disease entity, protean in character as is syphilis, undramatic in behavior, subtle in onset, and definitely nonbacterial in type. The protozoa are minute animal forms often tolerantly regarded by human tissue for a long time." Chronic amebiasis is nearly always an infection by the races with small cysts, and there is almost never a history of a dysenteric attack, rarely that of residence in the tropics or contact with people who might have brought back as convalescent carriers an amebic race that is known to have caused dysentery.

With matters standing thus, an added interest and a fresh turn given the discussion by an attack on the pathogenicity of the small races of *E. histolytica* by the author of the laboratory "bible" of parasitology, Professor Brumpt, who left the question doubtful in the last (1922) edition, while citing his own belief at the time as for the mutation of size races. In a preliminary note,¹⁵ he calls the attention of physicians to an "ameba generally confused with the ameba of dysentery, *Entameba dispar*."

The small race of *E. histolytica*, based on its wide diffusion throughout mankind and its being nondysenterogenic, set it aside as a new species, *E. dispar*, which Brumpt describes as resembling *E. histolytica* morphologically, in appearance, color, clear pseudopodia and faintly discernible nucleus, differing in the negative signs of the absence of the large motile forms, even after purgation, the absence of red blood cells in the food vacuoles, and slightly less motility at room temperature, all points previously noted by

Brumpt and others as characterizing the small race of ameba. The cyst is like that of *E. histolytica* in number and appearance of nuclei. *E. dispar* is ranked as a new species (a) on the basis of its food, bacteria and yeasts,* (b) its slight pathogenicity for the cat, in which it produces but a fugaceous lesion, never dysentery, and on a (c) second biologic ground of being widely distributed and not causing dysentery. He quotes statistics that show that in England 5 per cent of the population are carriers of the ameba with four nucleate cysts, in France 4 per cent, in the United States 5 per cent, in Venezuela 30 per cent, in Buenos Aires 24 per cent. Statistic studies show that the amebae with quadrinucleate cysts are as common in the stools of people inhabiting countries where amebic dysentery is rare as where it is frequent. Why is the ameba dysenterogenic for man once in four times in the Philippine Islands 1 in 20 in Macedonia, Indo China, the Senegal and Morocco, while in England with approximately 2 000 000 carriers but one or two autochthonous cases arise during the year?"

Sohci Shimura, a Japanese writer in 1918 noted the presence of an ameba "not pathogenic" for man in six individuals five with a normal intestinal tract, one with a nondysenteric chronic catarrh. With this ameba Shimura was able to give only a transitory lesion to 3 of 23 kittens inoculated, while he obtained positive results in 91 of 100 inoculated with amebae from dysenteric stools, and 50 in 100 making the kittens ingest cysts from dysentery. Similar experiments by Brumpt himself have changed his opinion expressed in the 1922 edition of the 'Parasitologie' that the small forms had a low infectious power, to the theory that the difference is that there is a non-pathogenic species. Brumpt and Drbohlav in the former's laboratory, using the technic of Boeck and Drbohlav† were able to infect kittens from healthy carriers. In thirty kittens Brumpt found no ulcerations only a few congested areas, although the amebae were extremely numerous, occasionally they contained red cells but there was no true dysentery.

The surprising epidemiologic facts are much easier to understand. Brumpt thinks if it is admitted that the widespread ameba is not dysenterogenic for man, 'perhaps not even pathogenic' and should be considered a species different from the dysenteric ameba the frequency of the latter having no relation to that of the quadrinucleate cysts. The morphology similar to that of the ameba of dysentery need not hinder our making the distinction 'for Prof. Dobell is incapable of distinguishing *E. ranarum* from *E. histolytica* either in the vegetative form or in cysts but separates it by biologic character'. Indeed, the statistics for amebiasis are being found to hold true for peoples like the Alaska Indians who have had no possible contact with the tropics and the difference between the number of carriers and the morbidity from dysentery is so great one might well be surprised. There is a difference of opinion as to the food of the small ameba. Only the large amebae ingest blood cells. The mode of nutrition says Dobell "is peculiar in this species, being mainly by absorption and bacteria are probably never ingested by nor

*In a case with diarrhea I studied with the small race of ameba 90 per cent contained bacteria, none blood cells.

†Rectal inoculation under ether anesthesia, and sealing the kitten's anus for twenty-four hours. Using this technic with amebae from two carriers with symptoms but who never had dysentery I was unable to obtain even implantation in young kittens.

morphology, slight in the higher organisms, more marked in the simpler. The change in size may be a mark of the change in character.

The differentiation of *Councilmania* by Kofoid and Swezy,¹⁸ has cleared a source of confusion, in the vegetative stage with *E. histolytica*, in cysts with *E. coli*. Similarly further differentiations may be expected in this new field of work. New staining methods, but chiefly, the cultivation of amebae, will enable clinicians to make more exact diagnoses. *E. dispar*, even if not a valid species, shows a partial fixation of character similar to that of the streptococcus races with joint, gall bladder, throat or other tissue localization. It is too early to draw any conclusions. Although enough facts may not have been adduced to prove *E. dispar* has a right to exist as a separate species, at least there are enough to show a tendency, in a protozoan parasite in the primate stock since its origin, toward the formation of a variety with fixed habits. But raising the question of the specificity of the ameba present in carriers, and Brumpt leaves it a question, will direct study by fixing attention upon definite points of attack in the ameba problem. More careful study of fecal protozoa is needed, far more than the customary separation of *E. coli* and *E. histolytica*. All species must be noted as found. We are still in the statistic stage with the protozoa, the stage of uncertainties, and of arithmetic errors, but a stage of hope. *E. dispar*, not mutable into a dysenterogenic species, or very exceptionally so, may exist, as species etiologic of much chronic illness. The mutability of the size races has yet to be proved. Between cultivation and animal experiments this may be determined, but human experiments may be necessary. A problem named is already half solved.

SUMMARY

1. There is evidence that the intestinal protozoa cannot be beneficial to man, only negative evidence that they are ever innocuous, and positive evidence that they can be dangerous, theoretically any of them might become pathogenic. The danger of an infection may be shown either by time of observation in the individual, or by the percentage of disease in a number of infections.

2. *Entameba histolytica* presents two types: a race with large cysts usually associated with amebic dysentery of tropical origin, and a race with small cysts that is common everywhere, rarely associated with dysentery, but apparently the cause of low grade illnesses in midlife. The most complete proof of the etiologic relationship of the ameba to this pathogenicity has been given by Kofoid and his followers.

3. Brumpt thinks the small race differentiated enough to be considered a separate species, *E. dispar*.

4. The question of mutability or immutability of the size races of *E. histolytica* awaits perfection of culture methods and media, its growth in pure culture free from bacteria, preferably with living tissue cells to approximate natural conditions. Study could then be made of the histolytic enzyme or other products. Surveys of different age-groups would show at what time of life amebic infection begins.

5 The effect of tropical climate on man and its possible reaction upon his parasites, suggested by Haughwout, is important Roddis and Cooper¹⁹ have shown a definite harm from tropical climate, lowered basal metabolism, blood pressure, suggesting the possibility of other variations

REFERENCES

- ¹Boyers, Kofoid, and Swezy Chronic Human Amebiasis, Jour Am Med Assn, Nov 7 1925, lxxxv, 1441-1447
- ²Haughwout Tissue Invasive Powers of the Flagellated Protozoa, Phil Jour Sci Septem-ber, 1918 xiii, 217-238
- ³Pollock and Pickard Protozoal Infections of the Intestines, Am Jour Med. Sci April, 1919 clvii, 492
- ⁴Barrow Significance of Flagellate Infection in Certain Chronic Diseases Calif State Jour Med, October, 1921 xiv 395-400
- ⁵Mills Ocular Diseases Occurring in the Course of Vondy enteric Amebiasis Jour Am Med. Assn, Oct. 9 1926, lxxxvii 1176-1185
- ⁶Kofoid, Boyers, and Swezy Systemic Infection by Endamoeba Dysenteriae Proc Intern Conf. Health Problems United Fruit Co Boston 1924 pp 381-391 (bibliography)
- ⁷Kofoid and Swezy Amebiasis of the Bones Jour Am Med. Assn, May 27 1922, lxxviii, 1602-1604
- ⁸Dobell and O'Connor The Intestinal Protozoa of Man Wood and Co New York, 1921
- ⁹Hegner and Tagliaferro Human Protozoology Macmillan New York 1924
- ¹⁰Brumpt Parasitologie (a new edition is to appear in 1924) Masson et Cie Paris
- ¹¹Crowell Treatment of Amebiasis Jour Am Med Assn July 7 1917 lxxxix 6-10
- ¹²Brown Endamoebiasis Jour Am Med Assn Feb 13 1926 lxxxvi 457-462
- ¹³Kofoid Discussion of Papers p 5-2 seq in United Fruit Co reference 6
- ¹⁴Kessel The Distinguishing Characteristics of the Parasitic Amoeba of Culture Rats and Mice Univ Calif Pub in Zool Feb 20 1924 xv 489-544
- ¹⁵Brumpt Etude Sommaire de l'Entamoeba Dispar u p Amibo à kystes quadrinucléés, parasito de l'homme Bull Acad Med Nov 3 1925 xciv No 35
- ¹⁶Simon Discussion Jour Am Med. Assn July 3 1926 lxxxviii 7
- ¹⁷Brown Endamoebiasis Jour Am Med Assn Feb 13 1926 lxxxvi 457-462
- ¹⁸Kofoid and Swezy Councilmanra Caffeuri, a Parasitic Ameba of the Human Intestine Univ Calif Pub in Zool, June 23 1921 xv 169-198
- ¹⁹Roddis and Cooper The Effect of Climate on Blood Pressure Jour Am. Med. Assn, Dec 18, 1926, lxxxvii 2053-2055

subcutaneous and muscular tissue, there is practically no fibrin present in the exudate. Sparse Gram-positive organisms having the morphology of both cocci and bacilli are scattered through the inflammatory area. Slight or moderate congestion with occasional minute hemorrhages occur in the medulla of the adrenals. In the other organs, moderate degrees of acute congestion are found.

The chief differences, therefore, from the lesions induced by *C. diphtheriae* are seen in the uniform occurrence of ulceration at the site of subcutaneous inoculation and the absence of diffuse hemorrhagic infiltration of the adrenals.

Guinea pigs inoculated intraperitoneally with a forty-eight hour broth culture of the *Corynebacterium ulcerans* die almost consistently from twenty-four to forty-eight hours later. At autopsy, a purulent exudate is usually found in the peritoneal cavity, as well as multiple small abscesses scattered through the omentum and liver.

The local reactions on rabbits resulting from both intracutaneous and subcutaneous injections of the microorganisms are always more severe than on the guinea pigs. The lesions are more extensive, and the more intense areas of induration, congestion, and especially necrosis are followed by the formation of an ulcer. White rats are less susceptible than guinea pigs.

TOXIN PRODUCTION

Broth cultures of this microorganism were filtered after seven days' incubation at 37° C. The veal infusion broth for these cultures is the same as that used in the preparation of diphtheria toxin. The potency of different lots of this filtrate varies, and there seems also to be a definite variation in the susceptibility of the guinea pigs to the toxin, since, at times, 0.1 cc injected intracutaneously may induce only a small area of induration and congestion, while again an extensive local reaction with an area of necrosis may result.

The filtrate is much more toxic for rabbits than for guinea pigs. Whereas 0.1 to 0.2 cc usually is required to induce a reaction about 1 cm in diameter in guinea pigs, 0.01 cc injected intracutaneously into rabbits results in a raised area of congestion and induration varying from about 2 to 2½ cm in diameter.

An injection of 2 to 3 cc of the toxin, subcutaneously, into guinea pigs may result only in the formation of an area of induration and congestion 3 to 4 cm in diameter, while, after an injection of 5 cc, a definite area of necrosis appears in the center of such a lesion. Occasionally, animals receiving the larger inoculum may die. The autopsy findings are similar to those of animals inoculated with living culture. The lesions induced by subcutaneous injections in rabbits are more severe than those in guinea pigs, 0.5 cc resulting in a marked local reaction.

Guinea pigs injected with even as much as 5 cc of the toxin usually survive, while a dose of 0.5 cc, either intraperitoneally or intravenously usually proves fatal for rabbits.

Mice injected with 0.5 cc subcutaneously or intraperitoneally survive, and no lesions are noted

The toxin is destroyed by heating at 56° C for ten minutes

IMMUNE SERUM

Since reactions were obtained which indicated that the organisms formed a soluble toxin, an attempt was made to produce an antitoxin by the immunization of a horse. This animal received inoculations of gradually increasing amounts of the toxin at three day intervals for eighteen months, except when trial bleedings were taken. An inoculum of 800 cc was used during the last eleven months. At the end of eighteen months, a serum was obtained 1/1,000,000 cc of which neutralized 0.01 cc of the toxin when injected intracutaneously into rabbits. Approximately 0.5 of a unit of diphtheria antitoxin neutralized this amount of the toxin, while 0.1 cc of normal horse serum had no neutralizing effect. Guinea pigs were protected against the local reaction induced by a subcutaneous injection of 5 cc of the toxin, when 1/1,000 cc of the antiserum was injected with it. Likewise, 300 units of diphtheria antitoxin protected against the reaction while 1 cc of normal horse serum gave no protection.

Before inoculation, the serum of the horse contained less than 1/1,000 unit of diphtheria antitoxin per cc. After immunization for thirteen months, however, approximately 30 units per cc were found to be present. Although immunization was continued the diphtheria antitoxic content of the serum decreased from this amount until there were approximately 5 units at the time the last serum was obtained from the animal.*

Although satisfactory protection was obtained against the specific toxin by the antiserum, the results were not so satisfactory when tested against cultures of the organism. When these cultures were tested intracutaneously in guinea pigs which had previously received 1 cc of the antiserum, the local reactions were only slightly diminished from those obtained on normal animals or on those which had received normal horse serum or 500 units of diphtheria antitoxin. When the twenty-four hour growth of the organisms from a Loeffler's blood serum slant was tested subcutaneously in an animal which had previously received 1 cc of the antiserum the local lesion was reduced from an extensive area of induration, congestion and necrosis of from 4 to 5 cm in diameter to one of induration and congestion about 2 cm in diameter. The degree of protection was not the same with all the strains studied for with some a small area of necrosis developed.

The antiserum was tested against cultures of virulent diphtheria bacilli as well as against diphtheria toxin and some protection was obtained since this serum contained as much as 30 units of diphtheria antitoxin per cc.

HUMAN TESTS

A few intracutaneous tests of the broth filtrate have been made on adults. Four people who had failed to react to the Schick test and were inoculated

*In the few instances in which the serums of horses which had been immunized with pneumococcus, streptococcus and tetanus toxins were tested for their diphtheria antitoxic content, there was little or no increase in the titer.

intracutaneously with 0.1 cc. of a 1:10 dilution of the toxic filtrate, developed definite areas of congestion, varying in extent. One such reaction, which measured approximately 18 mm. in diameter after twenty-four hours, had faded in the next twenty-four hours so that only a faint area of pigmentation remained. The other reactions were more extensive—one which measured 5×4.5 cm. after twenty-four hours had extended to 6.5 cm. in the long diameter after ninety-six hours, and marked pruritus was present.

Five people who had reacted definitely to the Schick test were given half the amount of filtrate. These, too, showed areas of congestion but not more extensive than those already described. Three of these faded, leaving a pigmented area after seventy-two hours, one remained more definite, while the others showed slight erythema for several days. All of these individuals failed to react to the heated filtrate.

The neutralizing properties of antitoxin for the toxin were tested in a few instances. The undiluted horse serum protected against an equal amount of a 1:10 dilution of the toxic filtrate, and a 1:20 dilution of the horse serum seemed to give almost complete protection against an equal amount of a 1:20 and a 1:10 dilution of the filtrate.

CLINICAL HISTORY OF CASES

In connection with this part of the work, it may be of interest to review the clinical histories of the patients from whom these thirty-one strains were obtained. Five of the cultures were isolated from cases diagnosed as diphtheria. These were submitted from various periods in the course of the disease, from thirteen weeks after onset in one instance to forty-eight hours in another. One culture was from a child about whose clinical condition no data were obtainable. Throat cultures from his sister contained virulent diphtheria bacilli. Six cultures were from patients with symptoms of a "cold" or tonsillitis, none of whom were ill over a few days. Eighteen cultures were from persons who were not ill, the specimens being collected in connection with surveys for carriers of *C. diphtheriae*. The tonsils in one case were considered enlarged, and, in another, it was stated that they were hypertrophied and diseased. One culture was sent for diagnosis, but, as in the other instances, no information concerning the clinical condition of the case could be obtained.

It may also be of interest to know that, about ten days after the preparation of one lot of toxin, one of the workers who had handled the cultures had a slight sore throat, and a culture of *Corynebacterium ulcerans* was isolated from it. The organisms persisted for about three weeks, although the throat was not sore after the first day.

DISCUSSION

A study of these cultures reveals a group of microorganisms resembling, in only a few respects, other diphtheria-like bacilli reported in the literature, except those of the "poison-producing diphtheroids" of Parker with which they appear to be closely allied. They differ somewhat from the latter in that the organism which he described did not have polar bodies and did not grow satisfactorily on ordinary media, luxuriant growth being obtained only when blood was present. The organism isolated in this laboratory grows well on plain agar, and polar bodies can be readily demonstrated. Parker does not record the reaction in gelatin. In general, the pathogenicity and toxin production of the cultures described by him correspond very closely to those studied here. He did not mention, however, that diphtheria antitoxin was present in the antitoxic serum. He stated that, whereas the necrosis induced

by intracutaneous inoculations of the microorganisms is conspicuous after twenty four hours, the reactions subside more rapidly than those induced by *C diphtheriae*, while in our experience the lesions may persist for a week or more

SUMMARY

The microorganisms here described differ from true diphtheria bacilli in the following respects

- 1 Rapid change in morphology from bacillary to coccoid forms usually in twenty four hours
- 2 Liquefaction of gelatin
- 3 Nonreduction of nitrates
- 4 Reaction from intracutaneous injection of microorganisms on both normal guinea pigs and on those immunized with diphtheria antitoxin
- 5 Formation of extensive ulcers on animals inoculated subcutaneously with a living culture
- 6 Production of a soluble toxin for which a neutralizing antitoxin was prepared. The serum of the horse thus immunized showed a definite, but limited, increase in its content of diphtheria antitoxin

Although the *Corynebacterium ulcerans* differs from the true diphtheria bacillus in these respects, it is doubtless a closely related species. From the evidence of pathogenicity that the cultures manifest it seems probable that it may be of etiologic significance in some of the inflammatory or ulcerative lesions, especially those of the nose and throat

For much of the technical work connected with this problem we are indebted to Miss H. H. Owen and Miss F. A. Fitzgerald. We also appreciate the assistance of Dr. C. A. Griffin in directing the tests for determining the diphtheria antitoxic content of the antiserum

REFERENCES

- ¹Klem, E. Pathogenic Microbes in Milk. Jour Hyg. 1901, 1, 8
- ²Mallory, F. B. and Medlar, E. M. The Etiology of Scarlet Fever. Jour Med Research. 1916, 1917, xxxv, 209
- ³Ruediger, E. H. The Occurrence of Virulent Pseudodiphtheria Bacilli in Gangrenous Tonsillitis and Pharyngitis of Scarlet Fever. Trans. Chicago Path. Soc. 1903, vi, 45
- ⁴Hamilton, A. The Question of Virulence Among the So Called Pseudodiphtheria Bacilli. Jour Infect Dis., 1904, 1, 690
- ⁵Mellon, R. R. A Study of the Diphtheroid Group of Organisms with Special Reference to Their Relation to the Streptococci. Jour Bacteriol. 1917, 11, 81
- ⁶Parker, F. A Group of Virulent Poison Producing Diphtheroids Isolated Especially from Postscarlatinal and Other Cases of Otitis Media. Jour Med Research. 1922, xliii, 387
- ⁷Orskov, J. Method for the Isolation of Bacteria in Pure Culture from Single Cells and Procedure for the Direct Tracing of Bacterial Growth on a Solid Medium. Jour Bacteriol., 1922, vii, 537

OBSERVATIONS ON THE RELATIONSHIP OF THE WASSERMANN REACTION, CELLS AND GLOBULIN CONTENT, AND THE COLLOIDAL GOLD PRECIPITATION REACTION OF SPINAL FLUIDS IN SYPHILITICS*

BY TH THJOTTA, M D , AND H SAETHRE, M D , OSLO, NORWAY

A COMPLETE examination of the cerebrospinal fluid inluetics consists usually of the following laboratory tests

Qualitative and Quantitative Globulin Increase Test

The Wassermann Reaction

Cell Count

Quantitative Estimation of Albumin

Colloidal Gold Precipitation Test

The variations of results of these individual tests in different clinical pictures of neurosyphilis have already been extensively reported by Nonne,¹ Eskuchen,² Pappenheim,³ Ravaut,⁴ Sicard,⁵ Jeanselme, Vernes and Block,⁶ With,⁷ Schou,⁸ and numerous other workers. It does not appear from a review of literature that the results of these individual tests have been comprehensively studied in comparison with the specific Wassermann reaction in neurosyphilitics, either before, during, or after specific treatment was begun. An inquiry into the relations between these laboratory tests inluetics presented the following questions: (a) To what extent is agreement or disagreement obtainable in the usually performed examinations of the cerebrospinal fluid? (b) Does any parallelism exist between the Wassermann reaction and other tests and what conclusions may reasonably be made as to the value of the Wassermann reaction in cerebrospinal fluids?

Material The material employed for comparison of the Wassermann reaction with other tests consisted of 200 cerebrospinal fluids, obtained partly from patients suffering with neurosyphilitic lesions, and partly from patients presenting the clinical picture of latent syphilis. No discrimination was exercised in the selection of the spinal fluids employed in these studies. Every specimen of spinal fluid sent to our laboratory during a definite period from the Clinic of Nervous Diseases at the Riks Hospital, and Department IV of the City Hospitals at Ullevaal, was included in these studies. The material was thoroughly examined by the five ordinary tests for cerebrospinal fluid and their reactions were collated in order to find any possible correlation between the results of such tests. Care was taken to record the results both qualitatively and quantitatively.

Our inquiry appeared to be of considerable interest to the clinician as well as the laboratory worker. It will always be useful to have on record any

*From the Army Bacteriological Laboratory and the Division of Nervous Diseases
Rikshospitalet, Oslo

Received for publication December 15 1926

definite correlation of serologic reactions in syphilis. Occasionally the simpler reactions may forecast the results of more extensive and complicated tests. The question often arises how large pleocytosis may a spinal fluid present before one may reasonably expect to find a positive Wassermann reaction, or in what concentration of globulin may one expect the Wassermann reaction to become positive? It is likewise of considerable interest to ascertain whether any relationship exists between a positive colloidal gold precipitation test and a positive Wassermann reaction, and again the question may be asked, which of these two reactions will first disappear and on which test ought the chief emphasis be placed when a spinal fluid should be considered normal?

Technic 1 The Wassermann reaction—The amounts of spinal fluid employed for the Wassermann reaction are always 10 cc, 05 cc, 02 cc, and occasionally 01 cc. To avoid unspecific inhibition in the spinal fluid, our routine has been to study spinal fluids without the addition of extracts. In a long series of investigations we never encountered specific inhibition and hence feel justified in stating that a fresh sample of spinal fluid, not contaminated by long standing never absorbs the complement in the Wassermann reaction by itself without the presence of extract.*

All the spinal fluids were examined without inactivation by heat, and in this respect unlike our routine with the blood sera. The extracts employed were prepared according to Kolmer⁹ partly from human and partly from ox hearts. After the alcoholic extraction of the muscle was completed, 02 per cent of cholesterol was added to one portion of the extract.

The performance of the Wassermann reaction was as follows. To the amounts of spinal fluid, in duplicate order were added the previously titrated dose of normal heart extract and cholesterolized heart extract, together with the similarly titrated dose of complement.

Different authors have claimed that the use of cholesterolized extract in spinal fluids was confusing on account of its tendency to self inhibition. Using only 02 per cent of cholesterol according to Kolmer⁹ and not 04 per cent, according to the German workers we never found self inhibition in the cholesterolized extracts strong enough to involve any risk. Hence, we disagree with those authors who are hostile to the use of cholesterolized extract in performing the Wassermann reaction in spinal fluids. On the contrary, this extract is as valuable in the Wassermann reaction in spinal fluids as it is in the Wassermann reaction of blood serum. We find that the two extracts give uniform results, although the cholesterolized extract gives a stronger absorption and as such gives a more protracted positive Wassermann reaction in the course of treatment than does the plain heart extract. It appears therefore, that the result of the Wassermann reaction performed with the cholesterolized extract must be finally relied upon for the decision whether a spinal fluid gives a positive or a negative Wassermann reaction under treatment.

After this paper was written we examined a spinal fluid obtained by puncture of the lateral ventricle in a case of cerebral tumor. This fluid gave complete complement absorption in doses of 10 cc, 05 cc, and 02 cc without nonspecific inhibition and with negative Wassermann reaction in the blood serum. A week later we received a sample of the patient's spinal fluid for examination. The Wassermann reaction was negative. Lues was not present and the reaction of the ventricular fluid was not specific, although it absorbed complement only in the presence of the Wassermann reaction extract. This case is the only instance on our records of nonspecific inhibition in the cerebrospinal fluid.

The incubation of our tests was for half an hour on the water-bath constant at 37°C , and reading was carried out immediately after hemolysis of the blood cells, i.e., when all the controls were thoroughly dissolved.

The Wassermann reaction was performed by this method in a number of spinal fluids from patients presenting the clinical picture of various nervous diseases of luetic and nonluetic origin. Agreement of the Wassermann reaction with the clinical diagnosis, makes it necessary to briefly summarize the material examined. This material contained beside the before mentioned 200 spinal fluids, 251 spinal fluids examined during the same period and sent from different sources to the Army Bacteriological Laboratory of Norway for the Wassermann reaction.

The 451 spinal fluids fall into the following groups: (a) 84 fluids from patients with nervous diseases probably of nonluetic origin. Among these, 83 gave negative Wassermann reactions, and 1 gave a positive Wassermann reaction. The only spinal fluid with a positive reaction came from a patient suffering with Ménière's disease (aural vertigo), the patient admitting a luetic infection twenty years previously. (b) 94 fluids were sent us from patients without clinical diagnoses. Among these, 88 gave negative and 6 positive reactions. Nothing further was known clinically about the 6 positive cases. (c) 116 fluids came from patients with manifest neurosyphilis. 98 of these gave positive Wassermann reactions and 18 gave negative reactions. Several of the latter occurred in patients specifically treated. (d) 157 fluids came from patients with lues, but without any known neurosyphilis, i.e., primary and secondary lues. Of these 150 were negative and 7 were positive.

These results argue favorably for the reliability of our tests. Negative Wassermann reactions were found in fluids from nonluetic lesions and a strong predominance of positive reactions from nervous lesions of known luetic nature. It was evident that such basis for comparison between results of the Wassermann reaction and other spinal fluid reactions must be established before any conclusions can be made.

2 Cell Count—The counting of cells was made in the Fuchs-Rosenthal counting chamber, and the whole volume, 3.2 cmm was counted. The cell count per cmm was always the one stated, as a third of the total count, such as $7/3$ cells per cmm representing a total count of $21/3$ cells for the 3.2 cmm . As far as possible, the counting was always made from the first portion of the fluid obtained by the spinal puncture. When blood occurred in the first portion, the counting was made in the subsequent clear portion of fluid. The counting of cells was always repeated twice, partly from the same pipette and partly from different pipettes. An average value was taken of the combined cell counts.

The authors do not propose to fix a limit for the normal cell count of a spinal fluid, but have only considered values between $0/3$ and $8/3$ as normal ones, and values of $9/3$ and above as pathologic. The opinions of various authors differ considerably as to what value should be considered the highest normal cell count. Thus Axel Neel claims $1/3$, Jeanselme and Chevalier $4/3$, Eskuchen, Holzemann and Pappenheim $15/3$ and Nonne $30/3$. The figure

probably lies lower than $8/3$, but for practical purposes the normal borderline should not be placed too low when dealing with luetic material

3 and 4 *Globulin and Albumin*—Globulin and albumin were examined according to the methods described by Bisgaard,¹⁰ Ross and Jones,¹¹ Boyd,¹² Turner,¹³ and Zalonecki.¹⁴ The total albumin was determined by a disc method on the principle of the Heller nitric acid reaction (28 per cent acid), the spinal fluid being diluted in normal saline solution and the reading being made in the apparatus of Bisgaard¹⁰ after three minutes. A dilution of 15 was considered pathologic since control tests in normal fluids show that the dilution in these never exceeded 10.

The globulin content was performed in the same manner using a solution of ammonium sulphate in place of nitric acid. If a qualitative test showed the presence of globulin, the fluid was diluted in saline solution and the result given as the dilution figure. A negative reaction was recorded as 0, and a positive reaction was considered pathologic.

5 *The Colloidal Gold Precipitation Reaction*—The colloidal gold precipitation was carried out according to the ordinary titrations of 1 10, 1 20, etc., up to 1 10 240. The colloidal gold was developed from 500 c.c. twice distilled water, 5 c.c. of 1 per cent gold chloride solution, 5 c.c. of 2.5 per cent glucose solution and the addition of a 2 per cent solution of potassium carbonate until the color of the colloidal gold was changed. The reactions were recorded in figures from 0 to 50. The colloidal precipitation reactions have partly been conducted by the authors and partly by Dr A. Folling of the medical laboratory at the Riks Hospital at Oslo.

To carry out a quantitative comparison between different reactions, it is necessary to obtain a standard, as homogenous as possible for determination of the degree of the reaction and difficulty was encountered in finding such a measure. We finally resorted to the use of such terms as 'weakly positive', 'positive' and 'strongly positive' reactions. It is necessary, then, to define what we understand by these terms.

On the subject of pleocytosis it was stated that values below $9/3$ were considered normal. Values between $9/3$ and $30/3$ were considered as "slight pleocytosis" or a 'weakly positive' reaction. Those between $31/3$ and $100/3$ simply as pleocytosis or a 'positive' reaction while all values above $100/3$ were considered as "strongly positive" reactions.

In the globulin reaction the dilution figure 10 was considered "weakly positive", 20 "positive" and all values above this figure as strongly positive reactions. For albumin the same scheme was used only that the dilution figures for albumin were 14-25 'weakly positive', 26-40, "positive", and all figures above 40, 'strongly positive'.

The colloidal gold precipitation was recorded as follows: a luetic curve was considered, "weakly positive," a tabetic curve "positive" and a paralytic curve "strongly positive."

The Wassermann reaction appeared to best advantage by the use of this scheme since the three dilutions were always employed. It was quite natural to consider a reaction in all three doses as "strongly positive," one positive

in 0.5 c.c. but not in 0.2 c.c. as a "positive" reaction, while those positive only in the large dose of 1.0 c.c. of spinal fluid as "weakly positive" reactions.

In the following tests, a spinal fluid was not considered normal without first showing the following characteristics: Cell count not above 8/3, globulin reaction negative, albumin not above 14, the colloidal gold reaction not above 1 and the Wassermann reaction negative.

Our material thus examined consisted of 69 cases of manifest neurosyphilis and 74 cases of latent syphilis. The 69 cases fell into the following three groups:

General paresis,	18 cases with 21 spinal punctures
Tabes dorsalis,	18 cases with 28 spinal punctures
Neurosyphilis of other types,	33 cases with 63 spinal punctures

The 74 cases of latent syphilis fell into two groups, namely:

Latent syphilis* with normal fluids,	35 cases with 35 spinal punctures
Latent syphilis* with pathologic fluids,	39 cases with 53 spinal punctures

TABLE I

RELATIONSHIP BETWEEN THE SPINAL FLUID TESTS AND THE WASSERMANN REACTION
A. AMONG UNTREATED CASES

WASSERMANN REACTION		CELL COUNT	GLOBULIN	ALBUMIN	COLLOIDAL GOLD REACTION				
DOSES IN C.C.	RESULT				CURVES	PER CENT	POS	NEG	NUMBER OF FLUIDS
0.1-0.2	++++	424/3 (++++)	3.5 (++++)	62 (++++)	Paralytic	77	100	0	22
					Tabetic	9			
					Lues	14			
					Normal	0			
0.5	+++ ++	234/3 (++++)	2.7 (++)	37 (++)	Paralytic	40	90	10	10
					Tabetic	20			
					Lues	30			
					Normal	10			
1.0	+	61/3 (+)	0.9 (-)	50 (++++) 26*	Paralytic	6	75	25	17
					Tabetic	13			
					Lues	56			
					Normal	25			
-	-	35/3 (+)	0.1 (-)	11 (-)	Paralytic	0	19	81	72
					Tabetic	4			
					Lues	15			
					Normal	81			

B. AMONG TREATED CASES

0.1-0.2	++++	57/3 (+)	1.56 (+)	32 (+)	Paralytic	48	100	0	25
					Tabetic	48			
					Lues	4			
					Normal	0			
0.5	+++ +	24/3 (+)	0.29 (-)	16 (+)	Paralytic	9	83	17	24
					Tabetic	52			
					Lues	22			
					Normal	17			
1.0	+	26/3 (+)	0 (-)	12 (-)	Paralytic	8	77	23	23
					Tabetic	31			
					Lues	38			
					Normal	23			

and explained in text
only positive reaction +++ and ++ = positive + = weakly positive and - =

on between early and advanced cases of latent syphilis does not play any
The only object of our study is to show the correlation of various reac-
of examination of the fluid and not to give a continued study of the
phases of syphilis

Thus, altogether, our material consisted of 143 cases with 200 spinal fluids

By studying the results of the examinations of 200 spinal fluids, a prominent difference was found to exist between the results of the examinations of fluids from untreated and from treated cases, particularly when individual reactions were compared. In Figs 1 and 2 recording separately the untreated and treated cases, this difference became very conspicuous. In regard to the untreated cases, it was seen that a negative or "weakly positive" Wassermann reaction corresponded to

- 1 General low cell count (left in Figs 1 and 2)
- 2 Low albumin content and slightly positive colloidal gold reactions (light or very lightly darkened circles, in Figs 1 and 2)

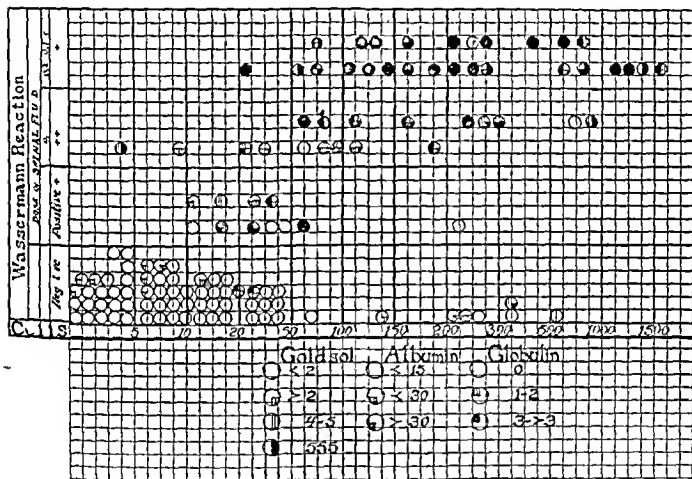


Fig 1—Untreated cases

It was noted in Figs 1 and 2 that as the circles with an increasing strength of the Wassermann reaction moved upwards along the ordinate, simultaneously was noted a transposition of the circles to the right, i.e., the cell count increased and the circles became more and more darkened i.e. the globulin (albumin) and the colloidal gold reactions increased in strength

In order to reduce the above facts into figures it becomes necessary to find an average value for the results of the examinations of all the fluids lying in the same graphic field. This was done in Table I

Table I A, dealing with the untreated cases showed that a "strongly positive" Wassermann reaction generally coincided with a very high cell count, strong globulin (albumin) and colloidal gold reactions while a "positive" and "weakly positive" Wassermann reaction occurred together with lower figures for the other reactions. This agreement between the reactions oc

curied in 83.2 per cent, while a larger or smaller disagreement was found in 16.8 per cent. To find a reason for the discrepancy between the various reactions was of the greatest concern to the authors in this inquiry. Disagreements between the Wassermann reaction and other reactions will be discussed below.

A positive Wassermann reaction was not found in a single case without the fluid also showing other pathologic reactions. In 49 out of 53 positive cases, at least two of the other reactions were positive. In this connection it must be mentioned that our material did not contain more than two cases of primary lues, while Schou,⁸ Fleischmann¹⁵ and other authors found a positive Wassermann reaction in the spinal fluid as the only pathologic reaction.

Considering the relation of the Wassermann reaction to the other reactions and starting with the cell count, we found that a "strongly positive" Wassermann reaction occurred with an average cell count of $424/3$, and

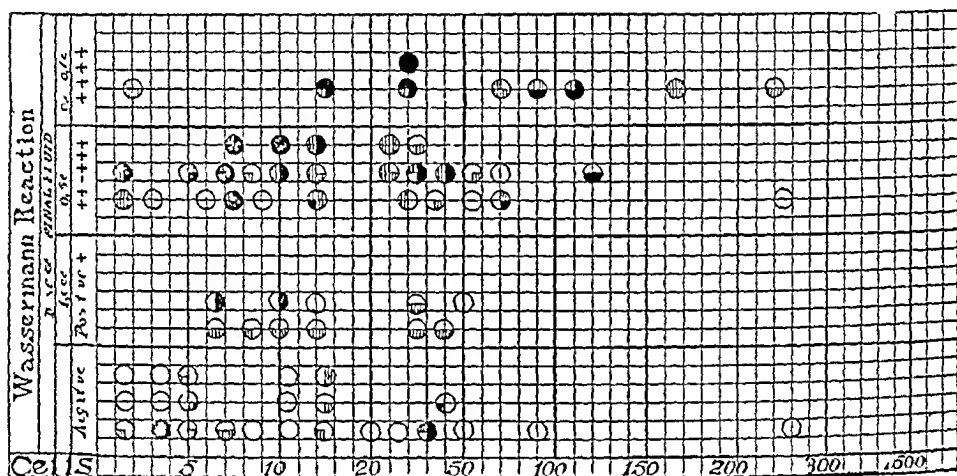


Fig. 2—Treated cases

varying at extremes between $1531/3$ and $53/3$. In a "positive" Wassermann reaction (dose 0.5 cc) the average cell count was $234/3$, varying at extremes between $510/3$ and $64/3$, while a "weakly positive" Wassermann reaction was found with a cell count of $61/3$, the maximum count being $234/3$ and the minimum $8/3$. A negative Wassermann reaction corresponded to a cell count of $35/3$, the highest figure being $503/3$, occurring in a case of transverse myelitis, and the lowest figure $0/3$. Only 8 cases were found to have a higher count than $50/3$ and in 7 of these no clinical signs of disease were found.

In the normal picture disagreements were sometimes found in excessively high cell counts. When such was the case in combination with a negative Wassermann reaction, it occurred usually in a spinal fluid syphilis without clinical symptoms or signs.

In three cases, however, the cell count was proportionally very low, namely, one case of tabes with $8/3$ cells, and two cases of cerebrospinal lues with respectively $12/3$ and $26/3$ cells. All these cases occurred in combina-

tion with a "slightly positive" Wassermann reaction. In the two last cases, the clinical symptoms were those of meningoaradiculitis and hemiplegia with paresis of the oculomotor nerve.

A high cell count without other positive reactions was found with only one exception in pure cerebrospinal fluid lues without clinical symptoms. The isolated pleocytosis cannot, therefore, be of any special importance. The very low cell count, however, was very seldom found together with a positive Wassermann reaction. This occurred now and again so that a low cell count cannot always be taken as an assurance of a negative Wassermann reaction, although under these circumstances as a rule one may expect a negative Wassermann reaction.

As regards globulin and albumin, the quantitative tests showed continually sinking values as the Wassermann reaction diminished in intensity. Thus, in a 'strongly positive' Wassermann reaction, the values were respectively 35 and 62, in a 'positive' Wassermann reaction, 27 and 37, in a 'weakly positive' Wassermann reaction, 09 and 50 and in a negative Wassermann reaction, 01 and 11. The high albumin figure of 50 in a slightly positive Wassermann reaction was found in two cases of subarachnoidal block, with abnormal albumin content. If we dismissed these two irregular cases, the proper figure was 27 instead of 50.

In disagreements between the Wassermann and globulin reactions, we twice encountered negative globulin reactions together with "strongly positive" reactions. One case was a patient with cerebrospinal syphilis occurring seven years after the luetic infection. The Wassermann reaction was strongly positive. The cell count 291/3, globulin and albumin respectively, 1 and 10, while the colloidal gold reaction showed 00121000000. The second case was in the spinal fluid from cerebrospinal syphilis with a positive Wassermann reaction, 510/3 cells, negative globulin, normal albumin and colloidal gold reactions. This patient was a man sixty years of age with an uncharacteristic dementia, miosis and dissimilar abdominal reflexes.

One might consider this an instance of incipient paralysis or a luetic arteriosclerosis. The cerebrospinal fluid reactions however ruled out encephalitis, disseminated, interstitial lesions or any considerable meningeal affection. In two other cases, we likewise found relatively too low values for globulin and albumin when compared with the Wassermann reaction. This picture was obtained in 4 out of 125 fluids.

On the other hand, we found in 5 out of 125 fluids a relatively too high value of the globulin and albumin reactions. This coincidence seemed to be of greater importance than the high cell count as we have found this condition only in cases of manifest neurosyphilis.

The colloidal gold reaction was never normal in fluids with the strongest positive Wassermann reaction. As was shown in Table I paralytic curves were found in more than three fourths of the fluids that gave a "strongly positive" Wassermann reaction. In positive Wassermann reactions, the paralytic curves were found in about 40 per cent of the fluids. On the other hand the fluids with negative Wassermann reactions never gave paralytic curves but presented normal curves in 81.5 per cent. The negative Wassermann reactions included

72 fluids of which 3 showed positive colloidal gold reactions, respectively, cerebrospinal lues with 12300000000 and with 02332000000 and hemiplegia with 12300000000. All the other curves were normal or approximately normal.

Treated Cases—The types and intensities of treatment have raised many questions as to results in spinal fluids, especially since the advent of malaria. It is a subject for another paper and not for this one to try to group results according to the type and duration of treatment.

While a striking parallelism existed between the results of the different reactions of the spinal fluids from untreated cases, the treated ones gave quite a different picture. By "treated" cases we understand cases that have been specifically treated with salvarsan or with bismuth in the course of the last six months previous to our investigation of their spinal fluids. Looking over the graphic scheme of the treated cases, we found that the transposition to the right side with the increasing strength of the Wassermann reaction is not quite as characteristic as among the untreated cases. The cell count was low as compared to the strength of the Wassermann reaction. By calculating the average values of the cell counts as mentioned above, we found a very prominent difference between the two kinds of patients. This was easily seen from Table I B. Corresponding to a "strongly positive" Wassermann reaction, we thus found only 57/3 cells against 424/3 in untreated cases, while the "positive" and "weakly positive" Wassermann reactions taken together corresponded to 24/3 cells and the untreated ones showed 234/3 and 61/3 separately.

The globulin and albumin figures likewise showed much lower values than did the same reactions in the untreated cases, which was easily seen from Table I B.

The colloidal gold reaction, however, presented a different picture, inasmuch as the fluids with a negative Wassermann reaction showed many pathologic curves. When the Wassermann reactions of medium strength were compared with the cell count and globulin-albumin reactions, there was likewise found a considerable number of pathologic curves. Among the Wassermann reactions of medium positive strengths, the positive colloidal gold curves were practically as numerous as among the untreated cases. This was easily seen from Table II, where the colloidal gold curves were tabulated.

Comparing the colloidal gold reaction curves with negative Wassermann reactions, a very prominent difference was found of 19.5 per cent positive curves in untreated cases, against 76.87 per cent positive curves in treated patients. Among these some were paralysis curves and about the same proportion of tabes and lues curves.

TABLE II
CORRELATION OF WASSERMANN REACTION AND COLLOIDAL GOLD PRECIPITATION REACTION

WASSERMANN REACTION	PER CENT POSITIVE COLLOIDAL GOLD REACTIONS	
	UNTREATED CASES	TREATED CASES
Strongly positive 0.2 cc	100	100
Positive 0.5 cc	90	83
Slightly positive 1.0 cc	75	81.4
Negative	19.5	76.87

Thus our material showed very distinctly that while the cell count and globulin albumin reactions were influenced in a relatively high degree by the treatment, the Wassermann reactions and colloidal gold reactions were very resistant, the latter especially being the most resistant to the course of anti syphilitic treatment

SUMMARY

Among 200 spinal fluids from syphilis, neurosyphilis, spinal fluid syphilis and latent syphilis, a considerable difference between treated and untreated cases was observed by a study of the ordinary spinal fluid reactions, such as the cell count, globulin and albumin reactions, the Wassermann and colloidal gold reactions

1 Among the untreated cases, 83.2 per cent showed a consistent agreement between the above mentioned reactions as regards the quality and degree of the reactions. The remaining 16.8 per cent of the fluids showed a larger or smaller disagreement between the various reactions. The causes of such disagreement were

a Most frequently a relatively too high cell count. This occurred in 8 out of 125 cases. In 7 of these the clinical diagnosis was the so called "spinal fluid syphilis" which may be due to an increase of lymphocytes in the cerebrospinal fluid.

b Less frequently it may be accompanied by a relatively high albumin content. The source of the 5 fluids wherein this condition was found, were cases of manifest neurosyphilis.

The colloidal gold reaction in no case occurred as an isolated pathologic reaction.

The Wassermann reaction was in no case found to be positive without one or several other reactions being pathologic. Among the 53 fluids with a positive Wassermann reaction, only 4 showed one single pathologic reaction beside the Wassermann reaction, while the 49 others showed several pathologic reactions.

In cases with strongly positive, positive and weakly positive Wassermann reactions, the cell counts were respectively 424, 234, 61 and with a negative Wassermann reaction, 35 cells. In one single case of tabes a positive Wassermann reaction was found combined with a normal cell count. On the other hand, 503 cells were found in a case with a negative Wassermann reaction in a male suffering from a transverse myelitis.

2 In 75 spinal fluids from treated cases agreement between the reactions was observed in only 34.67 per cent. In the other 65.33 per cent, there was a more or less pronounced disagreement.

a This disagreement was mostly caused by the low or very low cell count and albumin, simultaneously with a positive or strongly positive Wassermann reaction or colloidal gold reaction. Table I B showed that the various degrees of the positive Wassermann reactions corresponded to very much lower cell counts, globulin and albumin content following the treatment rather than before treatment was started. The colloidal gold reaction showed the same amount of positive curves before

amount of strongly positive reactions were found after treatment, when evaluated according to the results of the Wassermann reactions. In the presence of a negative Wassermann reaction, we found a greater number of positive reactions of the colloidal gold after treatment than before.

b In fluids from treated cases, the Wassermann and colloidal gold reactions may occur as isolated pathologic reactions individually or combined, while all other reactions may be completely negative. A positive Wassermann reaction was thus found twice in fluids from treated cases, otherwise the spinal fluid was completely normal. On the other hand, this combination was never observed among untreated cases.

In conclusion it may be said that the final criterion signifying that a spinal fluid in neurosyphilis has become normal during treatment, must be either a negative Wassermann or colloidal gold reaction, or better still, these two reactions occurring negative together. The finding of a negative globulin reaction, a normal cell count and albumin, will not suffice, however, as a means of telling whether or not a spinal fluid from a luetic is normal.

REFERENCES

- ¹Nonne, M. Syphilis und Nervensystem, Berlin, 1921.
- ²Eskuchen, K. Die Lumbalpunktion, Berlin, 1919, München med Wchnschr, 1922, lxx, 1536, *ibid*, 1923, lxx, 527.
- ³Pappenheim, M. Die Lumbalpunktion, Rikolverlag, Wien, 1923.
- ⁴Ravaut, P. Ann de dermatol et de syph, 1903, iv, No 1, p. 537, *ibid*, 1904, v, 1057.
- ⁵Sieard, M. Le liquide céphalo-rachidien, Paris, 1902, Compt rend Soc de biol, 1904, i, 357.
- ⁶Jeanselme, E., Vernes, A., and Bloch, M. Bull Soc dermatol et Syph, 1914, xxv, 12.
- ⁷With, C. Hospitalstidende, 1914, vii, 481, *ibid*, 1915, viii, 1151, Ugeskrift for Læger, 1916, No 32, p. 1353, Braun, 1918, xl, 403.
- ⁸Schou. Studier over den tidlige syphilitiske Meningitis, Copenhagen, 1922.
- ⁹Kolmer, J. A. Infection, Immunity and Biologic Therapy, Philadelphia, 1924.
- ¹⁰Bisgaard, A. Monatschr f Psychiat u Neurol, 1910, xxviii, 124, Ztschr f d g Neurol u Psychiat, 1912, viii, 381, *ibid*, 1912, x, 616, *ibid*, 1913, xiv, 329, *ibid*, 1913, xv, 508, "Eggehvidestofferno i Cerebrospinalvædsken, etc.," Copenhagen, 1913.
- ¹¹Ross and Jones. Brit Med Jour, 1909, i, 1111.
- ¹²Boyd, W. Jour Mental Sci, 1912, lvi, 203.
- ¹³Turner, J. Jour Mental Sci, 1910, lvi, 485.
- ¹⁴Zaloziecki. Monatschr f Psych u Nervenheilk, 1909, xxvi, 196, Deutsch Ztschr f Nervenheilk, 1913, xlvii xlviii, 783 (with critical summary of the literature).
- ¹⁵Fleischmann, R. Ztschr f d g Neurol u Psych, 1920, lx, 305, Ztschr f Nervenheilk, 1921, lxxviii lxx, 386.

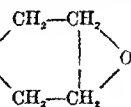
STUDIES IN TOXICOLOGIC CHEMISTRY*

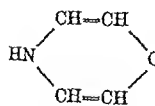
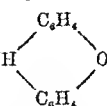
II THE FORMALDEHYDE SULPHURIC ACID REACTION OF THE OPIUM ALKALOIDS

BY VICTOR E. LEVINE, M.D., AND ESTELLE A. VAGIERA, M.D., OMAHA, NEBRASKA

THE chemical properties of the morphine molecule, its derivatives and the alkaloids closely associated with it, are interesting in view of the fact that these properties furnish the underlying reasons for many of the procedures at present employed in toxicology for their identification. Most of the tests for morphine and its derivatives relate to three of their conspicuous properties. One of these is their great avidity for oxygen. The other lies in their ability to lose a molecule of water on treatment with a dehydrating agent like hydrogen chloride or concentrated sulphuric acid. The resulting compound is apomorphine, which is morphine deprived of a molecule of water. The formation of this alkaloid is responsible for some very characteristic color reactions given by morphine, codeine and heroin. The third notable property of morphine and its associates is their reactivity relating to the presence of the phenolic group.

Morphine readily reacts with oxygen. Oxidation can be accomplished by atmospheric oxygen in the presence of alkali as well as by nitrous acid, potassium ferricyanide or ammonium copper sulphate. The first product of oxidation is oxymorphine or pseudomorphine ($C_{17}H_{19}NO_3$). This compound is non-toxic and is produced in the organism as a detoxication product of morphine. Further oxidation results in the formation of morpholin and fragments belonging to phenanthrene and possibly derivatives of naphthalene. These decomposition products possess chromogenic properties. Morpho-

lin, the formula of which is  is related to oxazine,

, and to the phenoxazine . The latter forms

the nucleus of well-known blue and violet coal tar dyes of determined constitution. The synthetic product, which Chastang named morpholin blue, is an example of one of the color compounds closely allied to morphine.¹

The ease of formation of chromogenic substances from morphine and its allied alkaloids explains why in their presence many oxidizing agents yield characteristic color reactions. Chlorine water colors morphine yellowish, chlo-

*From the Department of Biological Chemistry and Nutrition, School of Medicine, Creighton University, Omaha, Nebraska.
Received for publication May 6, 1926.

ine gas yields a deep yellow, ammonium hydroxide changes the color to red or brown. A solution of iodic acid in sulphuric acid colors morphine dark violet, then brown (Selmi¹). When morphine is heated with concentrated sulphuric acid and a drop of a solution of potassium chlorate subsequently added, a fine and long-enduring grass-green color is produced, while the borders of the liquid become faintly rose-red (Donath²). Mixing morphine with sulphuric acid and sodium arsenite results in the production of a dirty violet color changing to dark sea-green (Tattersall³). Morphine dissolved in dilute sulphuric acid yields a pale rose color with lead dioxide. Addition to the filtrate of ammonium hydroxide in excess produces a brown color, which persists for several hours. The color becomes transiently dark gray when the mixture is heated until white fumes are evolved. Morphine also reacts with nitric acid, and with a mixture of sulphuric and nitric acids (Husemann's reaction⁵). We shall consider later the reaction of nitric acid with relation to the phenolic properties of the opium alkaloids.

All substances that are readily oxidized have the ability to reduce compounds that give up oxygen with ease. That morphine is a great reducing agent is evident from the fact that it readily reduces gold and silver compounds even in the cold. In concentrated sulphuric acid it also reduces bismuth subnitrate with the production of a dark brown color.

One of the tests for morphine depends upon its behavior toward iodic acid. This latter compound is reduced to hydriodic acid. This acid easily decomposes in the presence of organic acid with the liberation of free iodine, which turns starch blue. The liberation of iodine from iodic acid is brought about promptly by many reducing agents, both organic and inorganic. The response of morphine to iodic acid is, therefore, not distinctive of this alkaloid. It is but an illustration of its reducing ability.

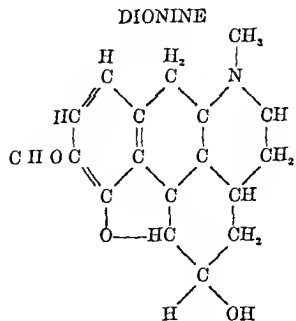
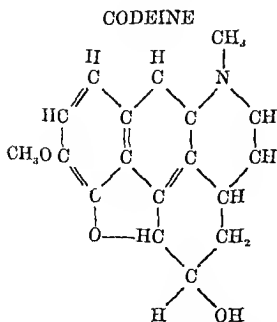
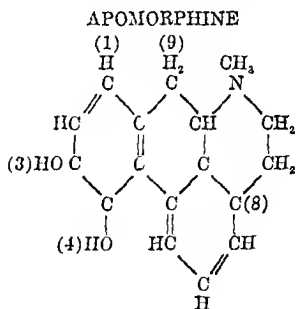
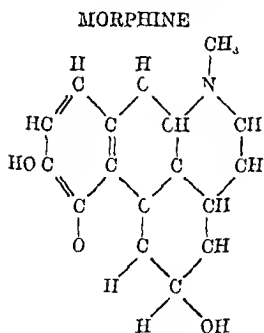
According to Lefort,⁶ the use of ammonium hydroxide after the addition of iodic acid results in the formation of a characteristic mahogany color. The ammonium hydroxide decolorizes the liberated iodine, and therefore permits the development of the color of the oxidation products of morphine. Lefort's test is believed to be distinctive of morphine and is regarded as having a positive value in proof of its presence. The iodic test without ammonium hydroxide has only a negative value. However, failure of reduction with the failure of formation of free iodine is proof of the absence of morphine.

Morphine also reacts with a mixture of ferric chloride and potassium ferrieyanide. The alkaloid brings about the formation of a blue color, the product resulting being ferrous-ferricyanide or ferric-ferricyanide or perhaps a mixture of both. Levine⁷ has recently pointed out that many organic compounds, especially phenols, reduce the ferric-ferricyanide reagent. However, the test has a negative value in establishing the absence of morphine, other reducing substances being excluded.

Another test based upon reduction is that involving the use of Frohde's reagent⁸ (molybdic acid in concentrated sulphuric acid) or Buckingham's reagent (sodium molybdate in concentrated sulphuric acid). Morphine is oxidized, while the molybdic acid is reduced, giving rise to compounds yielding colored solutions. The color produced with morphine is deep purple,

fading to violet, then changing to green Levine and Jahr⁹ have shown that a reagent similar to Frohde's or Buckingham's has a wide range of reactivity, giving positive results with aldehydes ketones, carbohydrates, amino acids, proteins, and other compounds Frohde's reagent gives bluish colors with codeine and narcein, greenish with apomorphine and berberine and reddish shades with brucine, emetine (changing to green) and yellowish tints with veratrine In connection with the reduction of molybdenum compounds, it may be added that the precipitate of morphine obtained by means of phosphomolybdic acid dissolves in ammonium hydroxide with a blue color

We now come to those tests bearing on the conversion of morphine into apomorphine. Solutions of the latter alkaloid assume a blue color when shaken with alkali. When morphine is converted into apomorphine, this color reaction with alkali develops (Deniges¹⁰). Gumbert and Leclère¹¹ have shown that the same reaction may be produced with greater rapidity and intensity by boiling an apomorphine solution with sodium acetate and mercuric chloride. Deniges extended this reaction to morphine. A few milligrams of morphine with two or three drops of concentrated sulphuric acid are added to con-

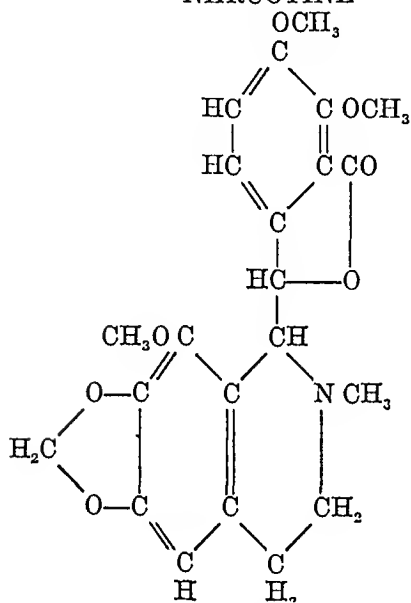


Heroin is diacetyl morphine, peronine is benzyl morphine

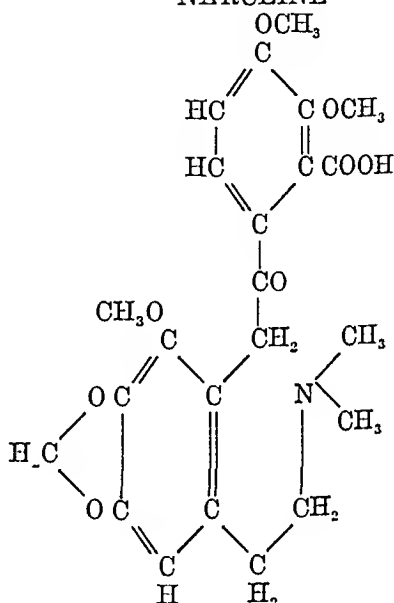
vert the morphine into apomorphine. The mixture is now diluted with 5 cc of a saturated solution of sodium acetate and two drops of 4 or 5 per cent solution of mercuric chloride. The whole mixture is boiled. Other alkaloids which can be converted to apomorphine—codeine, heroin and dionine—also respond to the test.

In the Pellagii reaction the alkaloid is first dissolved in fuming hydrochloric acid, concentrated sulphuric acid is next added, and the final mixture evaporated on an oil bath at 100° C to 120° C. Apomorphine, morphine, codeine, and heroin show purple at the edges. After the evaporation of the

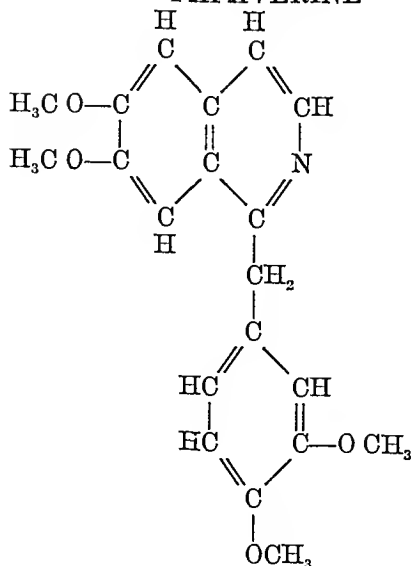
NARCOTINE



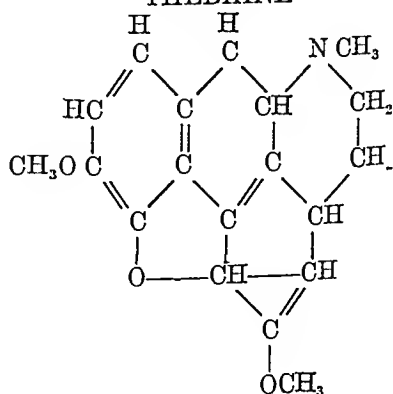
NARCEINE



PAPAVERINE



THEBAINE



hydrochloric acid, the purple assumes a red color. The latter changes to violet on re addition of hydrochloric acid followed by a neutralization with sodium bicarbonate. If hydriodic acid or a dilute solution of iodine in alcohol is now added, the color changes to green and dissolves in ether, which it tints purple.

Morphine possesses three oxygen atoms. One is alcoholic, another is phenolic, and the third is indifferent, forming the so called bridge oxygen atom. Codeine is a phenolic ether, methyl morphine. Dionine is ethyl morphine. Heroin is diacetyl morphine, peronine is benzyl morphine. Apomorphine is made from morphine by the removal of a molecule of water as a result of dehydration with concentrated hydrochloric acid. It has two phenolic hydroxyls. Thebaine has two methoxy groups. Narcotine and narcaine, three papaverine, four. The formulas for the various alkaloids are given on pp 775 and 776.

Certain tests for morphine are directly referable to the presence in its molecule of the phenol group. Ferric chloride gives characteristic color reactions with many phenols. With this iron salt morphine yields a blue color, which is due to the reduction of the ferric salt to the ferrous state and to the oxidation of morphine. It is very likely that the main color product is a substance closely bound up with the phenol group. It is to be remembered that phenols other than morphine—gallic, tannic and salicylic acids—also yield blue colors with ferric chloride.

The reaction with nitric acid or with sulphuric and nitric acids is typical of phenols. Concentrated nitric acid dissolves morphine with an orange red or deep red color, finally changing to yellow. The yellow color is due to reactions analogous to those taking place in the formation of yellow picric acid or trinitrophenol from phenol. In the Husemann test morphine is dissolved upon a watch glass in a few mills of concentrated sulphuric acid. The colorless solution is heated for thirty minutes on the water bath, until white fumes arise. The mixture is allowed to cool and two or three drops of concentrated nitric acid are added. A fugitive reddish violet color appears, soon changing to blood red, yellowish red or yellow and gradually disappearing. Potassium nitrate may be substituted for nitric acid. Oxydimorphine, apomorphine, codeine, and heroin react like morphine. Levine and Hammett¹³ have shown that monophenols, diphenols, triphenols and also derivatives of these types, react with nitric acid in the presence of sulphuric or phosphoric acid to give, as a rule, a red or reddish purple color.

Uranium nitrate (5 per cent) in alcohol or aqueous solution and neutralized with ammonium hydroxide gives, according to Lamal,¹⁴ with morphine in not too dilute a solution, an orange or red color. Phenol with uranium acetate also yields a red color (Orlow¹⁵).

Still another test which seems to depend upon the presence of the phenolic group is Lautenschlager's diazonium test.¹⁶ Morphine readily forms dye stuffs with diazonium compounds. A red color is produced on the addition of diazotized sulphanilic acid to morphine in alkaline medium. Under the same conditions sparteine yields a yellowish color, conine and nicotine a bright yellow, emetine and physostigmine, a red color. Of the alkaloids that

react at all with the Lautenschlager procedure, only morphine is stable in acid solution, although the red color formed changes to orange on the addition of acid. The fact that no other opium alkaloid except morphine reacts to Lautenschlager's reagent leads to the belief that the free phenolic group is cause for the positive response, since no such free group exists in codeine (methyl morphine), diomum (ethyl morphine), heroin (diacetyl morphine), narceum, narcotine, thebaine, or papaverine.

Very recently another test has been added to the long list of tests already known for the morphine alkaloids. Ekkert^{16a} in 1926 described a color reaction for morphine based upon condensation with benzidine in presence of concentrated sulphuric acid. The test proved positive for morphine, methyl morphine, ethyl morphine, diacetyl morphine, benzyl morphine and apomorphine. Experiments, which we will report later, seem to indicate that the same reagents react also with non-alkaloidal phenols.

THE REACTION SYSTEM, PHENOL-ALDEHYDE-ACID

Many color reactions employed in biochemistry result from the interaction in the system, phenol-aldehyde acid. The phenol and aldehyde condense to form a colored product. By the use of this system, characteristic tests may be obtained for any one of three components in the system: (1) aldehydes or compounds yielding aldehydes on oxidation (alcohols, such as methyl alcohol and glycerol) or on decomposition (carbohydrates, aliphatic carboxylic acids, such as oxalic, succinic, malic, tartaric, citric, glycollic, and lactic acids), (2) phenols of all types, (3) inorganic acids, such as hydrochloric or sulphuric acid. Chloroform, bromoform, and iodoform can also be tested for by this triple component system, for these halogen derivatives react readily with sodium or potassium hydroxide to yield the sodium or potassium salt of formic acid. This acid is a carboxylic acid which may be regarded as also possessing an aldehyde grouping.

TEST FOR ALDEHYDES

To test for aldehydes some of the well-known reagents used consist of a phenol and an inorganic acid possessing dehydrating properties like hydrochloric or sulphuric. The reagents, phenol and a dehydrating acid, together with the aldehyde-containing substance to be tested, complete the reaction system, phenol-aldehyde-acid.

The table gives a few tests for formaldehyde. These tests can also be employed for the detection of methyl alcohol, when on oxidation it is made to yield formaldehyde.

TABLE I—FORMALDEHYDE

PHENOL	ACID	END RESULT
Phenol	H ₂ SO ₄	Red ring
Resorcin	H ₂ SO ₄	Red color
Naphthoresorcin	HCl	Flocculent precipitate, darkening on standing
Guaiacol	H ₂ SO ₄ and FeSO ₄	Violet ring
Phloroglucin	HCl	Finely divided precipitate, solution becoming orange in color
Pyrogallol	H ₂ SO ₄	Chocolate brown color
Galic acid	H ₂ SO ₄	Green ring, changing to deep blue ring

TESTS FOR GLYCEROL

The system, phenol aldehyde acid, can be employed for the detection of glycerol. When this trihydric alcohol is oxidized by chlorine or bromine, it yields dihydroxyacetone and eventually the aldehyde methyl glyoxal. After oxidizing the glycerol the excess of bromine or chlorine is driven off. An alcoholic solution of orcinol, resorcinol or codeine is used together with concentrated sulphuric acid to give the reactions shown in the table.

TABLE II—GLYCEROL

PHENOL	ACID	END RESULT
Orcinol	H ₂ SO ₄	Beautiful violet or greenish blue
Resorcinol	H ₂ SO ₄	Wine red color
Codeine	H ₂ SO ₄	Beautiful greenish blue on heating

In the Mandel Neuberg^{12b} test for glycerol oxidation is brought about by means of sodium hypochlorite. The aldehyde glycerose which is formed, is made to react with orcinol in the presence of hydrochloric acid to give a beautiful violet or green blue color.

TESTS FOR ALIPHATIC CARBOXYLIC ACIDS

The following acids—oxalic, citric, succinic, tartaric, malic, glycollic, lactic—decompose on treatment with strong sulphuric acid to yield formaldehyde or the aldehyde acid, formic acid, which decomposes to form carbon monoxide and water. Owing to their characteristic decomposition these acids readily respond to tests with reagents containing a phenol and concentrated sulphuric or hydrochloric acid.^{12c} The table following illustrates the reaction system, phenol aldehyde acid, applied to the testing of some aliphatic carboxylic acids.

TABLE III—ALIPHATIC CARBOXYLIC ACID

ALDEHYDE PRODUCING ACID	PHENOL	INORGANIC ACID	END RESULT
Succinic acid	Resorcin	H ₂ SO ₄	Yellowish red solution with green fluorescence
Malic acid	β naphthol	H ₂ SO ₄	Blue color changing to green on heating
Tartaric acid	β naphthol	H ₂ SO ₄	Intense blue color
Tartaric acid	Resorcin	H ₂ SO ₄	Bright red color
Tartaric acid	Pyrogallol	H ₂ SO ₄	Fine violet blue color
Citric acid	β naphthol	H ₂ SO ₄	Greenish yellow changing to bright yellow on heating

Since oxalic acid decomposes on treatment with concentrated sulphuric to form the aldehyde acid, formic acid, it also responds to reactions resulting from its interaction with sulphuric acid and a phenol. According to Demges,^{12d} p cresol, guaiacol, and codeine can be used as reagents for the detection of glycollic or lactic acid. Glycollic acid decomposes on treatment with sulphuric acid to yield formaldehyde, while lactic acid under similar treatment yields acetaldehyde and formic acid.

TESTS FOR CHLOROFORM

Chloroform, bromoform, and iodoform react with potassium hydroxide to form potassium formate. Formic acid in its structural composition contains an aldehyde grouping. This fact argues for the possibility of utilizing the

system, phenol-aldehyde-acid, as a means of detecting the halogen compounds mentioned. That this is the case is evident from the Schwarz resorcinol¹⁶⁰ test and the Lustgarten naphthol test for chloroform, bromoform or iodoform.

TABLE IV—CHLOROFORM

NAME OF REACTION	PHENOL	ALKALI	END RESULT
Schwarz	Resorcinol	NaOH	Yellowish red color attended by a beautiful yellowish fluorescence
Lustgarten	α or β naphthol	KOH	Evanescient blue color, changing in contact with air to green, then to brown

In the above tests alkali is used instead of acid. The alkali is used to decompose the trihalogen derivatives. That acid can take part in the reaction is shown by the fact that the blue solution obtained with Lustgarten's reagents can, on acidification, be converted into a red dyestuff.

TESTS FOR FURFURAL

Furfural is an aldehyde obtained on the decomposition of carbohydrates with hydrochloric or sulphuric acid. The tests in the table following illustrate the use of the system, phenol-aldehyde-acid, for the purpose of detecting furfural.

TABLE V—FURFURAL

NAME OF REACTION	PHENOL	ACID	END REACTION
Mohlsch	α naphthol	H_2SO_4	Reddish violet zone
Bial	Orcinol	HCl	Green solution or a green flocculent precipitate

TESTS FOR CARBOHYDRATES

Inorganic acid, sulphuric or hydrochloric acid, and a phenol are reagents used to test for carbohydrates. The acid reacts with the carbohydrate to yield furfural or a derivative thereof. The formation of the aldehyde, furfural, completes the reaction system, phenol-aldehyde-acid.

TABLE VI—CARBOHYDRATES

NAME OF REACTION	TEST FOR	PHENOL	ACID	END RESULT
Mohlsch	All carbohydrates, glucosides and glucoproteins	α Naphthol	H_2SO_4	Reddish violet zone
Seliwanoff	Ketone sugars	Resorcin	HCl	Red color and separation of brown red precipitate on heating
Tollens	Glycuronates	β Naphthoresorcin	HCl	After heating, mixture extracted with ether, which assumes a violet red color
Tollens	Pentose, galactose, glycuronates	Phloroglucine	HCl	Red color
Bial	Pentose	Orcinol	HCl and $FeCl_3$	Green color and a green flocculent precipitate

TESTS FOR FREE INORGANIC ACID IN GASTRIC CONTENTS

The reaction system, phenol-aldehyde-acid, is a useful one in detecting inorganic acid, like hydrochloric or sulphuric. Boas' test and Gunzberg's test

serve to detect free hydrochloric acid in gastric contents. The reagents used, phenol and aldehyde, complete the triple system, phenol aldehyde acid.

TABLE VII—FREE ACID IN GASTRIC CONTENTS

NAME OF REACTION	PHENOL	ALDEHYDE	END RESULT
Boss	Besorcinol	Cane sugar (yielding furfural with acid)	Rose red color after heating
Gunzberg	Phloroglucin	Vanillin	Purplish red color after heating

TESTS FOR PHENOL

For identifying phenols it is necessary to use a mixture of aldehyde and acid. Meizer's reaction¹⁷ for phenol, Morner's reaction for tyrosine, a phenolic amino acid, Pettenkofer's¹⁸ or Udrinsky's¹⁹ reaction for morphine are illustrations of the system phenol aldehyde acid, applied to the qualitative analysis of phenols.

TABLE VIII—PHENOLS

NAME OF REACTION	TEST FOR	ALDEHYDE	ACID	END RESULT
Meizer	Phenol	Benzaldehyde	H ₂ SO ₄	Violet blue color
Morner	Tyrosine	Formaldehyde	H ₂ SO ₄	Green
Udrinsky	Morphine	Furfural	H ₂ SO ₄	Purple, changing to blood red
Pettenkofer	Morphine	Sucrose (yielding furfural with acid)	H ₂ SO ₄	Purple, changing to blood red
	Oxymorphone	Sucrose	H ₂ SO ₄	Green changing to blood red
	Codeine	Sucrose	H ₂ SO ₄	Purple, changing to blood red

TESTS FOR PHENOLIC ALKALOIDS

In 1896, Marquis²⁰ first reported his test for the phenolic alkaloids, depending upon the color formed with formaldehyde in the presence of sulphuric acid. The Marquis test seems to fit in with the scheme of the phenol aldehyde acid reaction. The phenols are the opium alkaloids while the aldehyde and the acid are contained in the reagent.

TABLE IX—PHENOLIC ALKALOIDS

PHENOLIC ALKALOID	ALDEHYDE	ACID	END RESULT
Morphine	Formaldehyde	H ₂ SO ₄	Purplish red changing to violet and finally becoming blue
Oxymorphone	Formaldehyde	H ₂ SO ₄	Green
Codeine	Formaldehyde	H ₂ SO ₄	Violet
Apomorphine	Formaldehyde	H ₂ SO ₄	Violet
Narcotine	Formaldehyde	H ₂ SO ₄	Olive green changing to yellow

PHENOL-ALDEHYDE SULPHURIC ACID REACTIONS

MONOPHENOLS

Phenol -----raspberry red.
 o Cresol -----deep purple, deep red dark brown.
 (methyl phenol)
 m Cresol -----deep purple, deep red.
 p Cresol -----deep purple
 o-Xylenol -----greenish edges, dark red brown next day
 (dimethyl phenol)

m Xylenol	-----	reddish brown, changing in a few minutes to brown, in one half hour changed to olive green which persisted after a week
p Xylenol	-----	bright red or crimson changing soon to purple
Thymol	-----	reddish violet
Carvacrol	-----	reddish brown, slight tinge of brown around edges
α Naphthol	-----	slight reaction, very slight green, with brown tint predominant
β Naphthol	-----	olive changing to dark green

MONOPHENOLS WITH HALOGEN

Tribromphenol	-----	light red
Diiodothymol	-----	negative

NITRATED MONOPHENOLS

o Nitrophenol	-----	negative
p Nitrophenol	-----	negative
Dinitrophenol	-----	negative
Picric acid	-----	negative
(dinitrophenol)		
Picramic acid	-----	negative
(dinitro aminophenol)		

MONOPHENOLS WITH AMINO GROUP

Amidol	-----	dark blue
(1,3 diamino 4 hydroxybenzene dihydrochloride)		
Mictol	-----	slight violet coloration, slow reaction
(mono methyl p amido meta cresol sulphate)		
Photol	-----	negative
(mono methyl p amido phenol sulphate)		

ETHERS OF MONOPHENOLS

Anisol	-----	purplish red
(methyl phenyl ether)		
Phenetole	-----	deep rose
(ethyl phenyl ether)		
Phenacetin	-----	negative
(acetyl derivative of p aminophenetole)		

MONOPHENOLS WITH ALCOHOL GROUP

Diathesin	-----	not soluble in reaction mixture, solid particles of the compound assume a lavender violet color, which persists even after two weeks
(o hydroxy benzyl alcohol)		

MONOPHENOLS WITH ALDEHYDE GROUP

Salicylic aldehyde	-----	blood red
(o hydroxybenzaldehyde)		

MONOPHENOLS WITH CARBOXYL GROUP

Salicylic acid	-----	carmine, lasting for more than three days
(o hydroxybenzoic acid)		
Aspirin	-----	carmine, very slowly changing to bluish
(acetyl salicylic acid)		

- β Oxynaphthoic acid -----green
 Tyrosine -----slow reaction, persistent characteristic deep blue developing
 (p hydroxy phenyl amino after long standing
 propionic acid)

MONOPHENOLIC ETHERS WITH CARBOXYL GROUP

- Anisic acid -----light green with lavender border
 (p methoxybenzoic acid)

ESTERS AND SALTS OF PHENOLIC ACIDS

- Sodium sulphocarbolate -----pink and red
 (sodium phenol sulphonate)
 Zinc sulphocarbolate -----purplish red, changing to green which persists for several days.
 (zinc phenol sulphonate)
 Sodium salicylate -----deep purplish red or carmine
 (oil of wintergreen)
 Methyl salicylate -----poor reaction evanescent purplish red streak in the yellowish
 reaction mixture
 Salol -----bright purplish red
 (phenyl salicylate)
 p Cresalol -----purplish red greenish around edges, after one half hour
 (p cresol salicylate) changes to persistent green

DIPHENOLS

- Pyrocatechol -----violet
 (o dihydroxybenzene)
 Adrenalin -----violet with a brown cast
 (o dihydroxyphenyl
 hydroxyethyl methylamino)
 Orcinol -----yellow changing at once to a bright raspberry red, which re-
 mains even at the end of three days
 Hydroquinone -----red brown with green tinge turning to muddy brown.
 (p dihydroxybenzene)

ETHERS OF DIPHENOLS

- Guaiacol -----purple
 (monomethyl ether of
 pyrocatechol)
 Eugenol -----dark reddish violet
 (allyl 4, 3 guaiacol)

ETHERS OF DIPHENOLS WITH ALDEHYDE GROUP

- Vanillin -----lemon yellow, changing to brownish red with purple green
 (m methoxy p hydroxy fluorescence
 benzaldehyde)
 Piperonal -----light olive green
 (also called heliotropino)
 (anhydride of vanillin)

ETHERS OF DIPHENOLS WITH CARBOXYL GROUP

- Vanillic acid -----deep orange red
 (m methoxy p hydroxy
 benzoic acid)

TRIPHENOLS

- Pyrogallol -----Indian red turning quickly to red brown.
 (1 2, 3 trihydroxybenzol)
 Phloroglucinol -----bright blood orange
 (1 3, 5 trihydroxybenzol)

TRIPHENOLS WITH CARBOXYL GROUP

Galic acid -----	purplish red changing to greenish, then to deep olive green (3, 4, 5 trihydroxy benzoic acid)
Tannic acid -----	evanescent yellowish green, poor reaction (digallic acid)

SALTS OF TRIPHENOLIC ACIDS

Dermatol -----	olive green changing to persistent dark green (bismuth subgallate)
----------------	---

GLUCOSIDES YIELDING PHENOLS ON HYDROLYSIS

Arbutin -----	slight green immediately changing to brown (yields hydroquinone on hydrolysis)
Salicin -----	purplish red, becoming blackish red on standing, with sul (yields saligenin or o hydroxy phuric acid alone—cherry red, becoming blackish brown on benzyl alcohol on hydrolysis) standing
Aesculin -----	persistent yellowish green

PHENOLIC ALKALOIDS

Morphine sulphate -----	purple red changing to violet and finally becoming blue
Oxycodone -----	green
Codeine sulphate -----	violet (methyl morphine)
Dionin -----	violet, changing to deep blue (ethylmorphine)
Heroin -----	violet (acetylmorphine)
Apomorphine -----	violet
Cotarnine -----	purplish red
Narcotine -----	olive green, changing finally to yellow

BITTER PRINCIPLES

Alcin -----	canary yellow, changing to red brown, same reaction with sul phuric acid alone
Crysarobin -----	with sulphuric acid red, which on standing changes to blackish brown, with reagent dark red, which on standing becomes purplish violet

COMPOUNDS OTHER THAN PHENOLS

α Naphthylamine -----	olive, changing to deep green
β Naphthylamine -----	very beautiful intense blue

The following compounds reacted negatively with the formaldehyde sulphuric acid reagent

Aldehydes—Paraformaldehyde, acetaldehyde, trichloroacetaldehyde, benzaldehyde, o nitro benzaldehyde, p dimethylaminobenzaldehyde, cinnamic aldehyde

Ketones—Acetone, chloroacetophenone, Michler's ketone

Alcohols—Methyl alcohol, ethyl alcohol, amyl alcohol, caprylic alcohol, glycerol, mannitol

Carboxylic Acids—Formic acid, acetic acid, butyric acid, caprylic acid, succinic acid, lactic acid, pyromucic acid, diacetic acid, palmitic acid, oleic acid

Amino Acids—Glycocoll, alanine, leucine, cystine, tryptophane, aspartic acid, asparagine

Proteins—Peptone gelatin egg albumin, collagen, osseomucoid.

Carbohydrates—Xylose, rhamnose, fructose glucose, mannose, maltose, lactose, sucrose, trehalose, raffinose.

Lipins—Olein, palmitin, stearin, lecithin, cholesterol

Miscellaneous Compounds—Benzene m dinitrobenzene, dimethyl aminobenzene, diphenylmethane, aniline, dimethylamine, p phenylene diamine, benzidine, semicarbazide, hydroxylamine, antipyrine, urea, thiourea, creatine, creatinine uric acid, iodoform, barbital, santal, sulphonal, trional, pilocarpine, cocaine quinine cinchonine, strychnine

The Marquis reaction is believed to be quite distinctive for the opium alkaloids and is regarded by Kobert¹ as the best at our disposal Gauss²² devised a colorimetric method for the estimation of morphine based upon this reaction

By reason of the fact that the reaction is of the phenol aldehyde acid type, there arises the possibility of extending the Marquis procedure to all phenols We have tested out the hypothesis and find that the test is applicable to all phenols examined—monophenols, aminophenols, phenol ethers, phenols containing an alcohol, aldehyde or carboxyl group, salts and esters of phenolic acids, diphenols, ethers of diphenols triphenols glucosides, and phenolic alkaloids The detailed results are given in tabular form

The colorations produced differ in most cases entirely from the phenolic alkaloids Some phenols of biologic occurrence however, produce effects similar to those given by these alkaloids The reactions obtained with pyrocatechin and para cresol are so similar to those of the opium alkaloids that it is impossible to make a distinction between them This fact argues for extra caution in interpretation of results It also detracts from the usefulness of the Gauss method for the colorimetric determination of morphine The method would be serviceable for the alkaloid in its pure form only, but in toxicologic mixtures or extracts it would be necessary to remove any phenols that react similarly to the alkaloids or any phenols that would throw off the color given by morphine For the sake of quantitative exactness it would also be necessary to remove any phenol, or any phenols that would modify the color given by morphine When a mixture of a phenol and morphine is made the color yielded with the Marquis reagent is a resultant of that given by the phenol and the alkaloid The color formed is not comparable to that given by pure morphine, thus impairing the accuracy of the colorimetric procedure devised by Gauss

Since the color tests for morphine and its associates lack specificity they should be used with great precaution as evidence of their presence Wormley²³ recovered morphine from the urine of opium patients from the blood and from fresh organs of animals by extraction with amyl alcohol and depended especially upon a positive Frohde reaction as a test for the presence of the alkaloid It is interesting to review some of the earlier medicolegal cases of morphine poisoning⁴ and to learn on what ground experts came to the conclusion that this alkaloid was the toxic agent used In 1871 Dr Medlicott was convicted in Kansas of murder by morphine and atropine An analysis of the cadaveric parts was made by the Stas method and affirmative results for morphine were obtained with the nitric ferric chloride, Frohde's and iodine

acid tests Atropine was determined by its reaction with sulphuric acid, and by physiologic tests Dr Kraus, of Tübingen, in 1878, reported the poisoning, in a German village, of a woman eighty-two years of age The poison was administered in coffee, which the deceased drank, notwithstanding its bitter unpleasant taste The symptoms were those of morphine poisoning, and death occurred within thirty-six hours The analysis was limited to the application of the iodic acid test

Another legal case was that tried in Portugal (1891-93) The defendant, *Caso Urbino de Freitas*, a physician, was accused of having poisoned three of his wife's nephews and of having caused the death of one of them by poison administered in enemas The symptoms resembled those caused by opium in part only The analysts claimed to have detected morphine, narcotine, and delphinine in the urine and in the viscera They based their conclusion as to morphine on the insufficient evidence furnished by the iodic acid, Frohde's reaction, and Lafon's reaction, the last one of which has recently been proved by Levine²⁵ to be characteristic not only of the morphine alkaloids but of all other compounds containing the phenolic group

It is evident from the above citations that the qualitative methods used were far from sufficient to yield conclusive evidence as to the presence of morphine In the case reported by Kraus and in the one reported by Wormley, neither the Frohde reagent nor the iodic acid reagent is specific, for a positive reaction may be given by any number of organic substances that possess reducing ability In the *Medlicott* case the nitric acid and the ferric chloride test could have been given by any number of phenols, while in the case of *Urbino de Freitas*, it has been very recently shown by one of us²⁶ that the Lafon test is a very reliable test for phenols in general It can also be said that even a combination of tests for phenol and for reducing power is not any more specific, for organic compounds there are, other than the morphine alkaloids, which have the ability to reduce and which also give a positive reaction for the phenol group

Since lack of specificity characterizes the color tests for the opium alkaloids it is not at all surprising to find that Vaughan,²⁸ in the *Buchanan* case in New York, pointed out that, while chemists for the prosecution swore to the presence of morphine and atropine in the dead body, all of the tests for the former made by the experts could be duplicated with the so-called putrefactive alkaloids The six tests relied upon by the experts for the state were the ferric chloride, the nitric acid, the Husemann, the iodic acid, the Frohde reaction, and the Pellagri reaction The ferric chloride, the nitric acid reaction, and the Husemann reaction (sulphuric and nitric acids) are phenolic reactions The Frohde reaction and the iodic acid reaction indicate merely reducing ability The only test that may at all be considered specific for morphine is the Pellagri test, which depends upon the conversion of the alkaloid into apomorphine The test is believed to be specific but like other tests for morphine, it may be found to be non-specific when subjected to re-investigation It must be remembered that in a putrefactive mixture of beef, Vaughan obtained a positive response with the Pellagri reaction, although no morphine was present Rosenbloom and Mills,²⁷ however, claim that bacterial products formed

during aerobic and anaerobic putrefaction of certain human organs did not in any way give reactions simulating those due to morphine

It is interesting to note that Vaughan^{21a} has finally stated that the possible sources of error in mistaking putrefactive reactions for those of morphine have been definitely removed. This improvement has resulted from the greater purity of extractive reagents used and from better analytic methods. With reference to possible error due to putrefactive products, we may add in passing that van Itallie and Steenhauer have in 1925 reported that bases are formed by the action of bacteria. Some of these bases are precipitated by the usual alkaloidal reagents. Some of them even give color reactions resembling those of alkaloids. The investigators, van Itallie and Steenhauer,^{2b} report that, in the toxicologic examination of a portion of liver from an exhumed cadaver, they isolated a compound which gave certain reactions for veratrine, but which proved to be the phenolic compound, p hydroxyphenyl ethylamine.

In the light of our newer knowledge we must strengthen our evidence as to the presence of morphine in cadaveric material. It is very possible for a toxicologist to base his conclusion as to the presence of morphine upon five or six tests, every one of which represents but one characteristic property of this alkaloid, and which may also be possessed by non alkaloids. It is for this reason that we strongly emphasize the fact that every toxicologic analysis for morphine should include at least one test from each of the six groups representing five distinct chemical properties and one biologic property characteristic of morphine and its associated alkaloids. The groups are given below.

Group I Tests involving precipitation by the so called alkaloidal reagents, which are general for all alkaloids

- a. Phosphomolybdic acid
- b. Iodine in potassium iodide
- c. Potassium bismuth iodide
- d. Potassium mercuric iodide

With the last precipitating reagent Koller² devised a microchemical test depending upon the formation of yellow star shaped or broom shaped crystals or spherocrystals.

Group II Tests involving the reduction of the reagents used

- a. Iodic acid
- b. Ferric chloride and potassium ferricyanide
- c. Molybdic acid in concentrated sulphuric acid (Froehde reagent)

Group III Tests involving oxidation of the alkaloid.

- a. Nitric acid
- b. Nitric and sulphuric acids (Husemann reaction)
- c. Iodic acid and ammonium hydroxide (Lefort reaction)

Group IV Tests depending upon the presence of the phenol group

- a. Selenious acid and sulphuric acid (Mecke reagent)
- b. Furfural and sulphuric acid (Udránsky reagent)
- c. Formaldehyde-sulphuric acid (Marquis reagent)
- d. Diazonium reaction (Lautenschlager reagent)
- e. Benzidine and concentrated sulphuric acid (Ekkert reagent)

ACTION OF IRON AND ALUMINUM SALTS ON ENZYMES

In 1904 I⁶ found that the digestion of starch was inhibited by $N/333 \text{ FeCl}_3$ while it required $3N/10 \text{ AlCl}_3$ or about 100 times as strong as the non solution. It is possible that some correction in this should be made for hydrogen ion concentration, which may not have been sufficiently considered at that time. This, however, would not alter the fact that in general iron is more toxic than aluminum.

ACTION OF IRON AND ALUMINUM SALTS ON THE DEVELOPMENT OF FISH EMBRYOS

Mathews⁷ found that $M/12000 \text{ FeCl}_3$ or $M/20 \text{ FeCl}_2$ killed and prevented the development of fertilized fundulus eggs, while a concentration of $M/9 \text{ AlCl}_3$ was required. This result indicates that aluminum chloride is only about one-half as toxic as ferrous chloride and over one thousand times less toxic than ferric chloride.

THE ACTION OF IRON AND ALUMINUM SALTS ON THE GERMINATION OF CLOVER SEEDLINGS

R. W. Ruprecht⁸ compared the toxicity of iron and aluminum salts in the development of clover seedlings. His results show that ferrous sulphate exerts a toxic effect when it is present in over four parts per million. Aluminum sulphate was found to be toxic when present in the culture solution in concentrations greater than forty parts per million. According to this aluminum is ten times as toxic as ferrous sulphate which is much less toxic than ferrous salts. While these concentrations of aluminum prevent the germination of plants, more dilute solutions of aluminum have been found by Stoklasa⁹ to be beneficial.

TOXICITY OF IRON AND ALUMINUM SALTS TO GOLDFISH

$M/20$ solutions of ferric chloride and aluminum chloride were prepared and a goldfish placed in each solution. The iron chloride solution killed the goldfish in an average of twenty-five minutes while in the aluminum chloride solution the fish lived approximately two hours.

ACTION ON FROGS

Hans Meyer and Francis Williams¹⁰ investigated the toxicity of iron in 1880. They found that 5 to 10 mg hypodermically in frogs caused a complete paralysis after twelve to twenty-four hours. I have found that iron is much more toxic than aluminum to frogs.

TOXIC ACTION ON DOGS

Kunkel¹¹ says that the deadly intravenous dose of iron is 20 to 50 mg per kilo body weight while the toxic dose of aluminum is 150 to 300 mg per kilo, or an average of 225. According to this, iron is six to eight times as toxic as aluminum. I have been able to confirm these figures. I found that the toxic dose for dogs when molecular solutions of the drug were injected intravenously was

Ferrie sulphate	30 mg per kilo
Aluminum potassium sulphate	200 mg per kilo
Aluminum chloride	234 m _g per kilo

Rabbits, cats and dogs were uniformly paralyzed and killed in twenty four hours by doses of 30 to 60 m_g of iron per kilo intravenously

Hans Meyer and Williams¹ found that 0.6 gm of ferrous sulphate injected into the veins of a dog caused pronounced vomiting and diarrhea. Eight gm given by stomach caused the death of a dog in twenty six hours. Postmortem examination showed ecchymosis of stomach and intestines.

ACTION WHEN GIVEN BY MOUTH

Numerous feeding experiments on both men and animals have been performed with aluminum compounds and traces may be absorbed which apparently follow the routes of iron in the body.

Leary and Sheib¹³ fed dogs and rats very large amounts and observed no ill effects, except possibly the death of one rat while receiving 300 mg per day of aluminum. They conclude that their experiments have no special bearing on the question of aluminum compounds as used in the diet of man largely because the amounts used could not possibly obtain under any condition in man. With rats the ingestion of aluminum amounted to 300 to 3000 mg daily per kilo of body weight.

ACTION ON MAN

The question of toxic action of aluminum in man was investigated by Chittenden, Taylor and Long,¹⁴ who found that aluminum compounds furnished in foods up to 150 mg per day for several months were without influence on health. No aluminum was found in the blood or urine. Winter Blyth¹⁵ and his family used alum baking powder for months without any apparent injury. Iron compounds however as used in medicine may exert a decidedly toxic effect.

Meyer and Williams¹⁶ quote Frank as stating that 28 to 40 gm of ferrous acetate taken internally over a period of fourteen days caused great inclination to sleep, often colic and pains in stomach, vomiting and diarrhea. The blood was darker than normal and the heart beats were reduced to about one half the normal.

LETHAL DOSE OF ALUMINUM AND IRON FOR MAN

The fatal dose of aluminum compounds is not definitely known. Blyth figures that the lethal dose of aluminum for a man of 68 kilos (150 pounds) is about 17 gm or 3 ounces of ammonium alum. Death has been reported following the ingestion of 45 cc or 1½ ounces of the tincture of iron which is equivalent to about 6 gm or a dram and one half of the salt.¹⁷

ACTION ON VITAMINES

It has been stated that metals may have an effect on vitamins, also that toxic agents may exert the action first on the germ cells. This is highly questionable and the reverse may be true. Recently Manville¹⁸ has shown that vitamin B deficiency may manifest itself for the first time in the second

- ¹⁰Deniges Med Chron, 1919, xix, 49
- ¹¹Grimbert and Leclère Jour Pharm Chem, 1915, vi, 23
- ¹²Pellagri Gaz chim ital, 1877, vii, 297, Jour Chem Soc, 1897, xxxii, 808
- ¹³Levine and Hammill Unpublished communication
- ¹⁴Lamal Jour Chem Soc, 1895, lvi, 11, 375, Semana méd, 1894, xiv, 267
- ¹⁵Orlow Pharm Jour, 1920, vi, 267
- ¹⁶Lautenschlager Arch d Pharm, 1919, cclvii, 13
- ^{16a}Ekkert Pharm Zentral, 1926, lxxvii, 498
- ^{16b}Mandel and Neuberg Bioch Zeit, 1915, lxxv, 214
- ^{16c}Weston Scheme for the Detection of Carbon Compounds, Longmans, Green and Co, ed 3, 1912, p 31
- ^{16d}Denigés Bull Soc Chim, v, 647, Bull Soc Pharm Board, xix, 50
- ^{16e}Autenrieth Laboratory Manual for the Detection of Poisons, Philadelphia, Blakiston's Son and Co, 5th American ed, 1921, p 36
- ¹⁷Melzer Ztschr f anal Chem, 1898, xxxvii, 345
- ¹⁸Pettenkofer Medical Jurisprudence, Forensic Medicine and Toxicology, New York, 1911, ed 2, Wm Wood and Co, p 995
- ¹⁹Udráusky Ztschr f anal Chem, 1888, vi, 355
- ²⁰Marquis Dissert Dorpat, 1898, Arb der pharm Inst zu Dorpat, 1896, vi, 117
- Prescott Pharm Arch, 1901, iv, 89, Jour Soc Chem Indust, 1898, viii, 954
- Hurdusheka and Faul Arch d Pharm, 1917, ccliv, 171
- ²¹Kobert Apoth Ztg, 1899, xxxvii, 259
- ²²Gauss JOUR LAB AND CLIN MED, 1921, vi, 699
- ²³Wormley Univ Med Mag, 1890, ii, 399, Chem News, 1890, lxx, 65, 79, and 99
- ²⁴Witthaus and Becker Medical Jurisprudence, Forensic Medicine and Toxicology, New York, 1911, ed 2, Wm Wood and Co, p 995
- ²⁵Levine JOUR LAB AND CLIN MED, 1926, xi, 809
- ²⁶Vaughan and Novy Cellular Toxins, Philadelphia and New York, Lea Brothers & Co, 1902, p 240
- ²⁷Rosenbloom and Mills Jour Biol Chem, 1913, xvi, 327
- Rosenbloom Jour Biol Chem, 1914, xviii, 131
- ^{27a}Peterson, Haines, and Webster Legal Medicine and Toxicology, Philadelphia and London, W B Saunders Co, ed 2, 1923, ii, 357
- ^{27b}Van Itallie and Steenhauw Pharm Weekblad, 1925, lvi, 446
- ²⁸Koller Schweiz Apoth Ztg, 1920, lxxviii, 300
- ²⁹Straub and Hermann Biochem Ztschr, 1912, xxxv, 216
- Kaufmann Asser Biochem Ztschr, 1913, li, 161
- Heinekamp Jour Pharm and Exper Therap, 1922, vi, 107
- ³⁰Marmé Zeit anal Chem, 1883, xiv, 642
- ³¹Balls Jour Biol Chem, 1927, lxxi, 537

THE PHARMACOLOGY OF IRON AND ALUMINUM IN RELATION TO THERAPEUTIC USES*

By H A McGUIGAN, M D, CHICAGO, ILL

THE chemical relationships of iron and aluminum are so intimate, that in many cases they are difficult to separate. Because of these relationships one should expect their pharmacology to be much the same. In general this is true.

Under some conditions both iron and aluminum are toxic. A study and restatement of some of the less known, but important actions are interesting and may lessen the misuse of iron therapeutically.

Aluminum is the most abundant of metals, being present in the earth's crust to the extent of nearly 8 per cent. One is surprised, therefore, to find

*Received for publication November 11 1926

traces only in most forms of animal life. Because of its absence in animal life, an unwarranted toxicity has sometimes been attributed to it.

It is probable that aluminum like iron is essential for life, especially of plants, although in many cases traces only are found. Apparently it is more important in some plants than in others. Maze believes that the presence of aluminum in the soil is necessary for the normal development of maize. Latshaw¹ found that the ash of maize contains 12.19 per cent aluminum and 9.74 per cent iron. Since practically all plants and all foods contain aluminum compounds either as essential ingredients or as contamination it is of importance to know the effect of aluminum when ingested by animals and man.

As is well known, iron is essential for the normal development of all green plants and perhaps all forms of animal life. The body of a man contains about 3.0 to 3.5 grams of iron, about 80 per cent of which is in the form of hemoglobin. Perhaps every cell in the body contains traces in an organic or nonionizable form. Aluminum is found, if at all, in traces only. Since iron is a normal constituent of the body and performs an important function, its administration is often looked upon as harmless and generally beneficial, while the absence of aluminum and a widespread feeling against its use makes a comparison of the actions interesting.

It is not generally known that iron is a poisonous metal and for this reason is often harmfully used in therapeutic attempts. The toxicity of aluminum on the other hand has been grossly overestimated due in part to the belief that it is a foreign element, and therefore necessarily toxic and to misinterpretation of the work of Siem and Doellken.² These investigators gave aluminum parenterally and found it to be toxic. Since, when given by mouth, it is absorbed at most in traces only a very limited application can be made of their investigation. In addition, they used mainly aluminum tartrate, and tartrates are also actively toxic when given parenterally.³ A restatement, therefore, is necessary.

IRON AND ALUMINUM IN COOKING UTENSILS

In 1913 the Lancet Laboratories⁴ conducted experiments to see whether or not sufficient aluminum from aluminum dishes was dissolved to be objectionable. They cooked a great variety of substances in aluminum and iron utensils and came to the conclusion that there is no evidence to show that in ordinary cooking either the iron or aluminum is so strongly attacked as to produce objectionable amounts of soluble salts. All that could be found after the use of organic acids or salts in the cooking were the merest traces of either metal. They conclude, therefore, that either metal is suitable material for cooking vessels.⁵

The case is different when an alkali is present. Carbonate of soda has no action on iron but it attacks aluminum freely and it is well to exclude carbonate or bicarbonate from all aluminum cooking utensils. In this respect it should be mentioned that the heating of alkali in glass will dissolve the glass so that the management of aluminum cooking utensils would require the same ordinary application of common sense as is necessary in the case of other metals for a similar purpose.

ACTION OF IRON AND ALUMINUM SALTS ON ENZYMES

In 1904 I⁶ found that the digestion of starch was inhibited by N/333 FeCl_3 while it required 3N/10 AlCl_3 or about 100 times as strong as the iron solution. It is possible that some correction in this should be made for hydrogen ion concentration, which may not have been sufficiently considered at that time. This, however, would not alter the fact that in general iron is more toxic than aluminum.

ACTION OF IRON AND ALUMINUM SALTS ON THE DEVELOPMENT OF FISH EMBRYOS

Mathews⁷ found that M/12000 FeCl_3 or M/20 FeCl_2 killed and prevented the development of fertilized fundulus eggs, while a concentration of M/9 AlCl_3 was required. This result indicates that aluminum chloride is only about one-half as toxic as ferrous chloride and over one thousand times less toxic than ferric chloride.

THE ACTION OF IRON AND ALUMINUM SALTS ON THE GERMINATION OF CLOVER SEEDLINGS

R. W. Ruprecht⁸ compared the toxicity of iron and aluminum salts in the development of clover seedlings. His results show that ferrous sulphate exerts a toxic effect when it is present in over four parts per million. Aluminum sulphate was found to be toxic when present in the culture solution in concentrations greater than forty parts per million. According to this aluminum is ten times as toxic as ferrous sulphate which is much less toxic than ferrous salts. While these concentrations of aluminum prevent the germination of plants, more dilute solutions of aluminum have been found by Stoklasa⁹ to be beneficial.

TOXICITY OF IRON AND ALUMINUM SALTS TO GOLDFISH

M/20 solutions of ferric chloride and aluminum chloride were prepared and a goldfish placed in each solution. The iron chloride solution killed the goldfish in an average of twenty-five minutes while in the aluminum chloride solution the fish lived approximately two hours.

ACTION ON FROGS

Hans Meyer and Francis Williams¹⁰ investigated the toxicity of iron in 1880. They found that 5 to 10 mg hypodermically in frogs caused a complete paralysis after twelve to twenty-four hours. I have found that iron is much more toxic than aluminum to frogs.

TOXIC ACTION ON DOGS

Kunkel¹¹ says that the deadly intravenous dose of iron is 20 to 50 mg per kilo body weight while the toxic dose of aluminum is 150 to 300 mg per kilo, or an average of 225. According to this, iron is six to eight times as toxic as aluminum. I have been able to confirm these figures. I found that the toxic dose for dogs when molecular solutions of the drug were injected intravenously was

Ferric sulphate	30 mg per kilo
Aluminum potassium sulphate	200 mg per kilo
Aluminum chloride	234 mg per kilo

Rabbits, cats and dogs were uniformly paralyzed and killed in twenty four hours by doses of 30 to 60 mg of iron per kilo intravenously

Hins Meyer and Williams¹ found that 0.6 gm of ferrous sulphate injected into the veins of a dog caused pronounced vomiting and diarrhea. Eight gm given by stomach caused the death of a dog in twenty six hours. Postmortem examination showed ecchymosis of stomach and intestines.

ACTION WHEN GIVEN BY MOUTH

Numerous feeding experiments on both men and animals have been performed with aluminum compounds and traces may be absorbed which apparently follow the routes of iron in the body.

Leary and Sheib¹³ fed dogs and rats very large amounts and observed no ill effects, except possibly the death of one rat while receiving 300 mg per day of aluminum. They conclude that their experiments have no special bearing on the question of aluminum compounds as used in the diet of man largely because the amounts used could not possibly obtain under any condition in man. With rats the ingestion of aluminum amounted to 300 to 3000 mg daily per kilo of body weight.

ACTION ON MAN

The question of toxic action of aluminum in man was investigated by Chittenden, Taylor and Long,¹⁴ who found that aluminum compounds furnished in foods up to 150 mg per day for several months were without influence on health. No aluminum was found in the blood or urine. Winter Blyth¹⁵ and his family used alum baking powder for months without any apparent injury.¹⁵ Iron compounds however as used in medicine may exert a decidedly toxic effect.

Meyer and Williams¹⁶ quote Frank as stating that 25 to 40 gm of ferric acetate taken internally over a period of fourteen days caused great inclination to sleep, often colic and pains in stomach, vomiting and diarrhea. The blood was darker than normal and the heart beats were reduced to about one half the normal.

LETHAL DOSE OF ALUMINUM AND IRON FOR MAN

The fatal dose of aluminum compounds is not definitely known. Blyth¹⁵ figures that the lethal dose of aluminum for a man of 68 kilos (150 pounds) is about 17 gm or 3 ounces of ammonium alum. Death has been reported following the ingestion of 45 cc or 1½ ounces of the tincture of iron which is equivalent to about 6 gm or 1 dram and one half of the salt.¹⁷

ACTION ON VITAMINES

It has been stated that metals may have an effect on vitamins, also that toxic agents may exert the action first on the germ cells. This is highly questionable and the reverse may be true. Recently Manville¹⁸ has shown that vitamin B deficiency may manifest itself for the first time in the second

generation If a metal had been given at this time it is probable that it would have been blamed for an effect on the germ-cells Again, metals, and especially aluminum, have been found to exert the opposite effect Daniels and Hutton¹⁹ draw the following conclusions from their investigation on the mineral deficiencies of milk

- 1 Rats fed exclusively on cow's milk seldom reproduce, and only a very small percentage of the young born survive
- 2 The nutritive deficiency of milk appears to be due to the fact that milk in low concentration, namely, manganese, fluorine, and aluminum, tion of a new generation
- 3 The addition to milk of those unusual mineral substances present in milk in low concentration, namely, manganese, fluorine, and aluminum, together with sodium silicate, has resulted in the production of five generations of normal young

THERAPEUTIC CONSIDERATIONS

As aluminum is used chiefly externally and for its astringent properties there is little likelihood of its misuse When taken by mouth in moderate amounts, there is little danger of toxic effect because traces only are absorbed There is no excuse for its use hypodermically and it is only when used in this way that it is of toxic importance

While iron also is used locally as a styptic, its main use is as a hematinic In view of the toxic effects recorded, it should not be given hypodermically unless the conditions demand it, and such an occasion is rare Most solutions of iron are precipitated at the site of action, hence are highly irritant Deep muscular injection does not lessen this effect but merely hides it If soluble salts which do not readily cause precipitation are injected they may be absorbed with sufficient rapidity to cause renal irritation or nephritis

In view of the toxic actions cited, and since there is no adequate evidence that the hypodermic use of iron salts is advantageous, the hypodermic method of administration of iron must be looked upon as inadvisable and often harmful

REFERENCES

- ¹Latshaw Jour Agric Research, 1924, xxvii, 845
- ²Siem and Doellken Arch f Exper Path u Pharm, 1898, vi, 98
- ³Pearce, R M, and Ranger Jour Med Research, 1913, xiii, 57
- ⁴Underhill, Wells, and Goldschmidt Jour Exper Med, 1913, xviii, 317
- ⁵Lancet, London, Jan 4, 1913, p 54
- ⁶Jour Am Med Assn, 1926, lxxxvii, 1397
- ⁷McGuigan Am Jour Physiol, 1904, v, 444
- ⁸Mathews Am Jour Physiol, 1904, v, 290
- ⁹Ruprecht, R W Mass Agric Exper Station Bulletin 161, 1915
- ¹⁰Stoklasa Biochem Ztschr, 1918, xci, 137
- ¹¹Meyer, Haus, and Williams, Francis Arch f Exper Path u Pharm, 1880, viii, 70
- ¹²Kunkel Handbuch der Toxikologie, 1898, pp 175 180
- ¹³Meyer, Hans, and Williams, Francis Arch f Exper Path u Pharm, 1880, viii, 70
- ¹⁴Leary and Sheib Jour Am Chem Soc, 1917, xxxix, 1072
- ¹⁵Taylor and Young Bull U S Dept Agric, 103
- ¹⁶Blyth, Winter Poisons, Their Effects and Detection, 1920, ed 5, p 723
- ¹⁷Meyer and Williams Arch f Exper Path u Pharm, 1880, viii, 70
- ¹⁸Peterson, Haues, and Webster Legal Medicine and Toxicology
- ¹⁹Manville Science, 1926, lv, 256
- ²⁰Daniels and Hutton Jour Biol Chem, 1925, lxxv, 113

LABORATORY METHODS

AN ACCESSORY TO THE CHAMBERS APPARATUS FOR THE ISOLATION OF SINGLE BACTERIAL CELLS*

BY WILLIAM H. WRIGHT, PH D., AND ELIZABETH F. MCCOY M.S., MADISON WIS.

THE ever increasing interest in the variability and pleomorphism of microorganisms has shown the need for the study of cultures derived from single cells. Many forms of apparatus have been developed for the isolation of small objects with the aid of the microscope. The method of Barber,¹ first briefly described in 1904 and in detail in 1914 made use of very fine hair like capillary pipettes. The pipettes were manipulated under a cover glass by means of the well known Barber apparatus. The preparation of a series of very small droplets containing bacteria on the under side of a sterile cover glass, as used in this method, is still the best procedure for organisms as small as bacteria.

The difficulties encountered in controlling the best constructed forms of the Barber apparatus prevented its general use by many bacteriologists. This has been true of other methods such as the one used by Topley, Barnard and Wilson.

The greatly improved double micromanipulator of Chambers² operating on an entirely different principle is capable of easy and accurate adjustment.

The use of the single Chambers apparatus with the Barber moist chamber and technique for the isolation of single cell cultures of bacteria has been described by Kahn.³ A single pipette is held in the micromanipulator which is clamped on the side of the microscope stage. A moist chamber 19 mm deep is used.

THE MODIFIED APPARATUS

A double form of the apparatus mounted on a heavy iron base with clamps for the microscope, as now made by the firm of Leitz leaves little to be desired in the way of rigidity or precision of adjustment. We have used this type of the apparatus for several months for bacteriologic work.

The control of evaporation is important in the formation of the tiny droplets used in the isolations. The manufacturers supply very satisfactory moist chambers in three depths with cover glasses to fit. The shallowest moist chamber (10 mm) lined with closely fitting strips of blotting paper on the sides has been found the most satisfactory. When saturated with distilled water these strips supply the moisture to the cover glass by evaporation.

*From the Department of Agricultural Bacteriology, University of Wisconsin.
Published with the approval of the Director of the Agricultural Experiment Station,
Madison, Wis.

Received for publication March 19 1917

and condensation. The shallow chamber has a small open end which checks excessive evaporation. It also permits the use of the ordinary substage condenser of the microscope.

For cell dissection and similar operations, there is an advantage in having the manipulator fixed in position in relation to the moist chamber and microscope. In bacteriologic work it is necessary to remove the bacterial cell

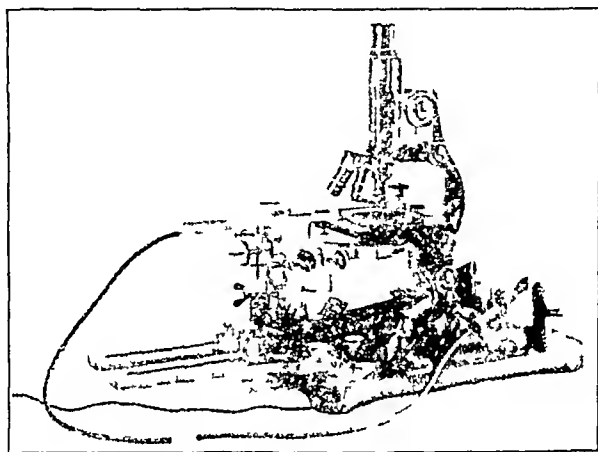


Fig 1—Modified form of the Chambers apparatus showing both of the micromanipulators in the working position

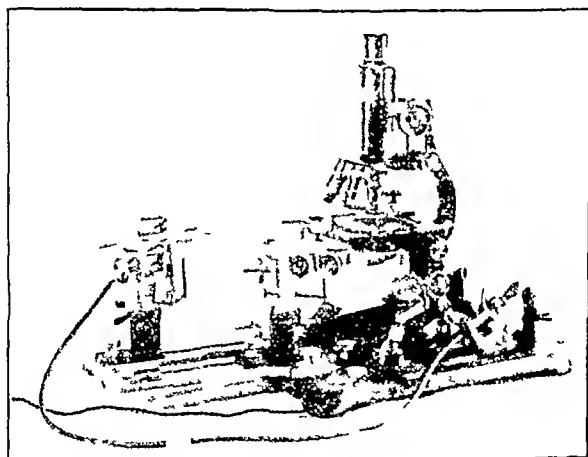


Fig 2—Modified form of the Chambers apparatus showing one micromanipulator in the distal position for removing pipette

from the moist chamber after it is picked from a droplet. With the original double form of the apparatus mounted on a base, the pipette with the single cell must be removed by hand. The narrow opening in the moist chamber as well as the compact nature of the manipulators present difficulties which may result in the breaking of the delicate tip of the pipette or in contamination of the culture.

It seemed to us that the apparatus could be constructed so that the entire manipulator with its pipette could be moved in a straight line and in the same

horizontal plane away from the moist chamber. Plans and suggestions were submitted to the manufacturers and they have constructed extensions for the base along which the entire manipulators may be moved. The modified apparatus is shown in Fig 1 with both manipulators in the working position near the stage of the microscope. When it is desired to remove a pipette from the moist chamber the locking screw on the base is loosened and the entire manipulator is moved back on its slide to the position shown in Fig 2. During the movement the pipette holds the same lateral and horizontal position it held while in the moist chamber. In this position it is a simple operation to remove the pipette or break off the tip in order to plant the culture.

The extensions for moving the manipulators away from the moist chamber also give the advantage of working with one apparatus without having the other one in the way. This allows much freer use of the vertical coarse adjustment than when both manipulators must be kept in the working position.

ISOLATION PROCEDURE WITH THE MODIFIED APPARATUS

- 1 Prepare a suspension of the organism or have a liquid culture ready
- 2 Moisten the strips of filter paper in the sides of the moist chamber and place it in the mechanical stage of the microscope
- 3 Clean a cover glass in cleaning solution and wipe dry with clean sterilized cheesecloth or gauze. We have found the most satisfactory cleaning solution to be one consisting of—

80 per cent ethyl alcohol	96 parts
Glacial acetic acid--	- 3 parts
Ether -----	1 part

Greasing treatment recommended by some to make sure of isolated droplets is not necessary. Neither is heat sterilization necessary as one can always see any bacteria there may be in the condensation droplets. In hundreds of observations we have never seen contaminated droplets.

- 4 A small loopful of the liquid culture or suspension is placed a few millimeters from one end of the cover glass near the center.

- 5 The cover glass is placed over the moist chamber with the hanging drop near the closed end.

- 6 The clamps are now loosened and both manipulators slid all the way out. A small microscope lamp like the Leitz 'Vignou' is centered on the mirror from the side and the light adjusted on the optical axis of the microscope. The heat from the lamp will help in the formation of the droplets. The rate of evaporation is readily controlled by varying the distance between the lamp and the mirror. In the 10 mm moist chamber droplets 2 or 3 micra in diameter will hold an hour or more without evaporation. Usually droplets tend to become confluent only in the closed end of the moist chamber near the large drop, when the heat from the lamp is too great or the cover glass too clean.

- 7, It is of advantage to work with as low magnifications as possible on account of the size of the field and light intensity. The color screens of the

"Mignon" lamp are also very helpful in getting the best illumination. A 15 X hyperplan or periplan eyepiece allows much work to be done with a 16 mm objective and a 4 mm gives the greatest magnification ever necessary.

8 The under surface of the cover glass is now brought into focus. This is easily done on account of the small dioplets in the field. By means of the mechanical stage the edge of the large drop is now brought part way into the field from the closed end of the moist chamber.

9 Pipettes as recommended by Chambers³ should be sterile and ready. These are best sterilized in a metal case by use of hot air. Rigid shank pipettes with tips at 90° and 45° angles are the most satisfactory. A 45° pipette is used to isolate single cells in dioplets and one of the 90° type to remove the cell from the moist chamber.

10 The 45° pipette is placed in the left hand manipulator which should be in the distal position, with the tip up and the pipette shaft horizontal. Be very careful not to make the pipette tip strike the cover glass or sides of the

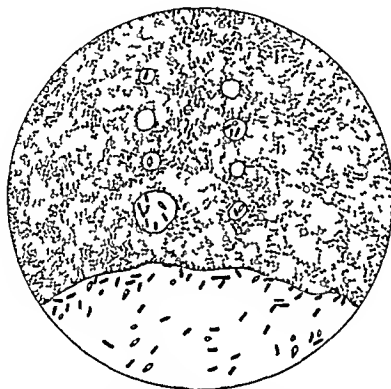


Fig. 3—A microscopic field showing droplets outside of the large drop. The very small droplets without bacteria are formed by condensed water vapor.

moist chamber, the entire manipulator is carefully moved into the working position or until the tip of the pipette appears in the microscopic field on the side opposite the large drop. This can be most easily done with the lowest magnification, viz., with the 16 mm objective or even with the eyepiece removed. Final lateral adjustments can be made with the manipulator screws when the eyepiece is replaced.

11 Vertical adjustment is easily made with the coarse adjustment. This adjustment may even be used for making dioplets on the under side of the cover glass.

12 With the tip of the pipette nearly touching the cover glass so that it is clearly in focus, the large drop is moved forward until it strikes the tip of the micropipette. The pipette will immediately fill with liquid and organisms. With the vertical adjustment the pipette is slightly lowered and the cover glass moved back by means of the mechanical stage. The pipette is now raised until it touches the cover glass when some of the liquid with the microorganisms will be seen to wet the cover glass. Upon lowering the pipette

a small droplet will be left with several organisms in it. This operation can be repeated again and again until parallel rows of droplets have been made, such as shown in Fig. 3. The first droplets usually contain several cells. The last ones may contain one or two or none. The adjustments are so easily controlled that a single cell can be transferred from one droplet to another with ease. The droplets formed by condensation are often convenient for this purpose as they are sterile.

13. When the cell selected is isolated in a droplet the manipulator carrying the 45° pipette is removed and the right hand manipulator with a 90° pipette is placed in position as before.

14. It is of advantage to have a length of small rubber tubing attached to the distal end of the 90° pipette and to a medicine dropper bulb, as shown in Figs. 1 and 2. If the bulb is slightly compressed at the time the tip of the pipette enters the droplet and then gently released the droplet and the cell will be sure to enter the pipette. In case the operator cannot control the bulb in a satisfactory manner with the fingers it can be done easily with the simple Hoffman screw clamp used by chemists.

15. By means of the mechanical stage the droplet and single cell are centered on the optical axis of the microscope. The 4 mm objective is now focused on the droplet with the tip of the pipette immediately under it. With the vertical fine adjustment the pipette tip is made to enter the droplet and the pressure on the bulb released. The bacterium may be seen to enter the pipette tip.

16. The pipette is now lowered to a safe distance below the cover glass and the micromanipulator slid to the distal position as before.

17. The tip of the pipette carrying the single cell is now broken off with a fine pointed sterile forceps and dropped into sterile culture media. Some workers recommend breaking off the tip of the pipette by pressing it against the side of the test tube in the culture medium. We have not found this very satisfactory because the natural break includes the shank of the pipette which has been exposed during the whole operation. It might be contaminated.

DISCUSSION

The use of the improved apparatus as described is no more difficult than the usual routine operations of a bacteriologic laboratory and takes much less time to get pure cultures. We have made as many as six isolations in thirty minutes. Three per hour is an average number.

The securing of single cells is no longer difficult. The much more difficult problem is to get more of the single cells to grow. This difficulty has been encountered by all who have undertaken to grow cultures from single cells of bacteria. Our results have been much like those reported by other investigators. We have been able to get growth from about 75 per cent of single cell yeast cultures, 33 per cent of spore bearing aerobes and only about 2 per cent of spore bearing anaerobes. Single spore isolations have given much better results than those of vegetative cells.

The growth of isolated single cells of bacteria may involve a quantitative relation of the cell to the volume of the culture medium or a mutual action of several cells on each other. In connection with the first hypothesis Robertson⁵ claims such to be the case for protozoa, although Cutler and Crump⁶ have not obtained the same results. With bacteria it is difficult to understand why a vigorous cell, motile at the moment of transfer, will not grow in some of the same medium from which it was isolated.

CONCLUSIONS

The modified Chambers apparatus makes the isolation of single cell cultures of bacteria simple and practical.

2 The simplicity of the procedure is of great advantage for the direct isolation of pure cultures from mixtures.

3 The low percentage of single cell cultures that grow, as shown by the number of sterile subcultures, is ample proof that contamination rarely occurs. Such results also indicate the need for more study of the conditions which influence the growth of single cells.

REFERENCES

- ¹Barber, M. A. The Pipette Method in the Isolation of Single Microorganisms and in the Inoculation of Substances into Living Cells, Philippine Jour. Sc., 1914, ix, No. 4, p. 307-358.
- ²Topley, W. W. C., Barnard, J. E., and Wilson, G. S. A New Method of Obtaining Cultures from Single Bacterial Cells, Jour. of Hyg., 1921, xx, 221-226.
- ³Chambers, R. New Micromanipulator and Methods for the Isolation of a Single Bacterium and the Manipulation of Living Cells, Jour. Infect. Dis., 1922, xxxi, No. 4, p. 334-343.
- ⁴Kahn, M. C. Chambers' Micromanipulator for the Isolation of a Single Bacterium, Jour. Infect. Dis., 1922, xxxi, No. 4, p. 344-348.
- ⁵Robertson, B. R. Allelostatic Effect in Cultures of *Colpidium* in Hay Infusion and in Synthetic Media, Biochem. Jour., 1924, xviii, 1240-1247.
- ⁶Cutler, D. W., and Crump, L. M. The Influence of Washing on the Reproductive Rate of *Colpidium colpoda*, Biochem. Jour., 1925, xix, No. 3, pp. 450-453.

NOTE ON THE TUNGSTIC ACID PRECIPITATION OF BLOOD PROTEINS*

BY MICHAEL SOMOGYI, PH.D., ST. LOUIS, MO

IN THE preparation of protein-free filtrates by the Folin-Wu method the precipitation of proteins is frequently incomplete, so that collection of the reaction by the addition of extra sulphuric acid becomes necessary. Since in V. C. Myers' book on blood analysis this difficulty is repeatedly mentioned, we infer that it is not confined to this laboratory.

In our routine work we have adopted Haden's¹ very convenient modification which consists in incorporation of the sulphuric acid in the water used for laking the blood so that 8 volumes of 1/12 N sulphuric acid are used in

*From the Laboratory of the Jewish Hospital.
Received for publication December 19, 1926.

the place of 7 volumes of water and 1 volume of $2/3$ N sulphuric acid. This certainly has the advantages of a more rapid filtration and a higher yield of filtrate but, naturally, does not obviate the above mentioned trouble. For this end we simply increased the concentration of the sulphuric acid above $1/12$ normal. According to Merrill² the nitrogen content of blood filtrates is unaffected by changes in P_H between 4.6 and 1. In our own experiments, however, much lower acidity than that corresponding to $P_H = 1$ already disturbs the precipitation. Thus, employment of $1/8$ N acid was found to cause considerable increase in the nitrogen content of the filtrate occasionally, and to give too low results in other instances.

The concentration of sulphuric acid we have ultimately chosen is $1/11$ normal as against the $1/12$ normal in the original Folin Wu proportions. This concentration proved to be not excessive in cases where the original amount of acid, too, was sufficient for complete precipitation, and was adequate when ever incompleteness of the precipitation according to Folin and Wu entailed the addition of extra acid.

TABLE I

NO OF SPECIMEN	$1/12$ N H_2SO_4		$1/11$ N H_2SO_4	
	NPN MG %	B. S. MG %	NPN MG %	B. S. MG %
200	10.2	169	35.7	168
268	28.6	101	30.5	106
305	30.2	84	28.7	97
306	40.8	87	37.8	86
313	33.7	—	24.7	—
315	34.4	—	34.2	—
325	29.6	117	41.4	110
326	30.0	97	2	86
327	36.4	94	16	90
329	31.0	—	20.0	—

Table I contains comparative determinations (picked at random as examples out of a greater number of experiments) of nonprotein nitrogen and sugar in filtrates obtained by the use of $1/12$ and $1/11$ normal sulphuric acid, respectively.

As can be readily seen the discrepancies are within the range of experimental errors.

As a result of our experiments the following procedure is recommended for the tungstic acid precipitation of blood proteins.

The blood is introduced into 8 volumes of $1/11$ normal sulphuric acid and after taking one volume of 10 per cent sodium tungstate is added. Shake allow to stand for about five minutes then filter.

REFERENCES

- ¹Haden, R. L. Jour Biol Chem 1923, lvi, 460
²Merrill, A. T. Jour Biol Chem, 1924, lx, 257

A COMPARISON OF THE QUANTITATIVE METHODS FOR THE BILIRUBIN OF THE BLOOD*

BY HOWARD F. SHATTUCK, M D, JOHN A. KILLIAN, PH D,
AND MARJORIE PRESTON, A B, NEW YORK CITY

EXPERIENCE within the last five years has demonstrated the diagnostic and prognostic value of the bilirubin content of the blood in the various types of jaundice. Although within the past eight years, considerable progress has been made in devising more exact technic for the bilirubin in blood serum, laboratory workers still are not agreed upon the most dependable and suitable method. Hence the results reported in the literature appear in variable terms and lack uniformity. Blankenhorn¹ in 1917 utilized a simple technic in estimating the bilirubin concentration of the blood serum. The blood serum was diluted to a point where the staining due to bilirubin was just perceptible in a column 1 cm deep. Blood sera requiring a dilution of 20 or more gave a positive Gmelin's test. In some instances of marked jaundice, dilutions as high as 275 were obtained. In 1921 Meulengracht² introduced a more exact technic, 0.5 cc plasma were diluted in a graduated tube with physiologic salt solution until the color matched a standard in a similar tube. The standard was a 1:10,000 solution of potassium dichromate containing two drops of sulphuric acid per 500 cc. Meulengracht stressed three possible sources of error, hemolysis, opalescence of lipemia and carotinemia. Normal blood plasma according to this method had a "plasma color" or "bilirubin number" of 1 to 5. In order to make the comparison with the standard more satisfactory, Gram suggested the use of blood serum instead of the plasma. Stetten³ reported the practical application to surgical problems of the results obtained by Bernhard and Maue in their studies of the bilirubin of the blood serum. Bernhard and Maue utilized a standard similar to that of Meulengracht, but made their comparisons of the blood serum against this standard in a plunger type of colorimeter. The depth of the standard solution divided by the depth of the blood serum required to match it gave a figure which they called the icterus index. Stetten reported the average normal icterus index of 3.6, and found that it may vary from 1.6 to 13.5 without clinical icterus. The threshold figures varied from 8 to 14. Bernheim⁴ has applied the icterus index determination to a study of the blood serum bilirubin in a variety of pathologic conditions. The normal range was found to be from 4 to 6, with a zone of latent jaundice extending from 6 to 16. Frank clinical jaundice was evident in all cases showing indices above 15.

We have in this modified Meulengracht test a simple direct means of measuring the intensity of color of the blood serum due to bilirubin. Bilirubin is not the only yellow-brown pigment of the serum. A part of the color of

*From the Department of Medicine and the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.

Received for publication Oct. 25, 1926.

the blood serum is due to luteins or lipochromes, pigments extensively distributed throughout the animal and vegetable kingdoms Meulengracht reports that in the determination of the 'plasma color' or 'bilirubin number' these pigments introduce an error of 1, a quantity which can be neglected for all practical purposes Carotin, a yellow brown pigment found in the vegetable kingdom and in animal secretions appears isomeric and perhaps identical with some of the luteins Carotin is found in human blood serum after the ingestion of meals containing vegetables Hess and Miers⁶ drew attention to the carotinemia in children on a diet of a high carotin content These authors noted the skin of the subjects were pigmented to such a degree as to simulate a mild jaundice However, the pigmentation was most evident on the palms of the hands, but the sclerae were not affected The carotin also appeared in the urine It is evident that the occurrence of a carotinemia introduces an error in the determination of the icterus index For this reason principally, the icterus index of the blood serum has been considered by some authors as unreliable in estimating the bilirubin of the blood serum Greene, Snell and Walters⁷ state that the icterus index may be used satisfactorily in following changes in the degree of jaundice in patients with frank icterus In such patients bilirubin is the preponderate coloring matter and changes in the serum color may reasonably be assumed to indicate variations in the amount of this pigment Conditions are different in specimens of serum in which the bilirubin is little if any increased over the normal In one case cited by these authors, the icterus index was 26 but the serum bilirubin was only 11 mg per 100 cc (normal) The effect of a carotin containing diet on the icterus index of the blood serum was studied by Bernheim⁸ Four normal subjects were given a meal containing carrots, three ate similar portions, and one ate a double portion Three hours later the icterus indices of the first three had been increased about 80 per cent above the control, while the fourth showed an index 250 per cent of his control The following morning all of the indices had returned to normal Errors due to carotinemia can easily be avoided by excluding from the diet all carotin containing substances for twenty four to forty eight hours, and by drawing the blood after a night's fast Obtaining the blood in the fasting state also obviates an unsatisfactory comparison due to postprandial lipemia Bernheim and Meulengracht have emphasized the precautions necessary to prevent hemolysis Preston⁹ has described the use of a disc standardized against the dichromate standard which has proved more satisfactory for comparison with blood sera, than has the dichromate solution Whereas the intensity of the color of the solution fades on standing exposed to light, the color of the disc is permanent The value of the icterus index of the blood serum in differential diagnosis in pathologic conditions involving liver function has been emphasized by Barrow Armstrong and Olds¹⁰ After an extensive study of the clinical value of some recent tests of liver function Shattuck Browne and Preston¹¹ reach the conclusion that the icterus index is the most useful single liver test that we have for clinical work

Lhrlich and Prosecki found that bilirubin enters into combination with an acid diazonium solution and van den Bergh utilized this reaction in a

quantitative method for bilirubin in the blood serum. The serum proteins are precipitated with alcohol and removed by centrifugation, the supernatant fluid is coupled with the diazonium solution and the color (reddish violet) compared with a standard. The standard used was either an alkaline alcoholic solution of bilirubin with the diazonium solution, or an ethereal solution of ferric thiocyanate. Schamberg and Brown¹² have found the quantitative method proposed by van den Bergh a delicate index of the changes of bilirubin in the blood serum. These authors have emphasized the value of the results obtained by this method as guides in the treatment of patients with arsenicals. Thannhauser and Andersen¹³ reported unsatisfactory results with this method because the unknown and standard did not match in the colorimeter. Moreover these authors have shown that the addition of the alcohol to the blood serum before the diazo reagents results in a loss of some bilirubin. Meulengracht has pointed out that precipitation of the serum proteins with alcohol introduces many errors. Part of the bilirubin is absorbed by the proteins and removed in centrifugation. A correction cannot be made for this loss because it varies greatly with different specimens. In some instances as in pernicious anemia, the loss was less than 5 per cent, but in cases of carcinoma of the pancreas, catarrhal jaundice and cholelithiasis the loss amounted to more than 50 per cent of the original concentration. In this connection it is interesting to note that Blankenhorn¹⁴ observed that in cases of acholic jaundice not all of the serum bilirubin was diffusible and this nondiffusible fraction did not pass into the urine. This retention and non-diffusibility of the pigment was due to a staining of the blood proteins by the pigment. The degree of staining varied with the concentration of the pigment and the length of time the plasma was exposed to the pigment.

Thannhauser and Andersen¹⁵ proposed a quantitative method for the serum bilirubin based upon the coupling of bilirubin with the diazonium solution. Their method essentially differs from van den Bergh's in that the diazonium solution is added to the blood serum before precipitation of serum proteins. After coupling of the bilirubin with the diazonium solution has been completed (color change becomes stationary) the proteins are precipitated by ammonium sulphate and alcohol. After centrifugation the color in the supernatant fluid is compared against a standard. A stock standard of bilirubin in chloroform is maintained and from this the azobilirubin in alcohol is prepared for use in the colorimeter. Results are reported in units of bilirubin, one unit being equivalent to 1 part of bilirubin in 200,000 parts of serum. This procedure is theoretically ideal, but practically impossible, since pure bilirubin cannot be obtained on the market. Greene, Snell and Walters have advocated Thannhauser and Andersen's technique for the color development in the blood serum, but use van den Bergh's ethereal solution of ferric thiocyanate as a standard. These authors state that it is impossible to check their standard against pure bilirubin but assume that it is equivalent to 0.2 mg of bilirubin per 100 c.c. Moreover it is admitted that the colors are not always identical, hence the comparisons are sometimes unsatisfactory. In 20 control cases without hepatic disease values from 0.3 to 1.4 mg of bilirubin per 100 c.c. of serum were obtained.

TABLE I

PATIENT	DATE	ICTERUS INDEX	BILIRUBIN		VAN DEN BERGH		CLINICAL JAUNDICE	DIAGNOSIS
			MC PER 100 C.C.	DIRECT	INDIRECT			
1 J M	2-3-26	33	0	-	+	0	Ethema of uterus 2ry anemia	
2 E B	1-8-26	60	pos	-	+	-	Typhoid fever, acute cholecystitis	
3 E V	2-19-26	60	pos.	-	+	0	Chronic cholecystitis	
4 G J	3-1-26	62	pos.	-	+	0	Chronic cholecystitis and appendicitis	
5 S B	8-29-26	37	pos	-	-	0	Myelogenous leucemia	
6 L S	1-29-26	71	pos.	-	+	0	Subacute endocarditis	
6 P C	3-1-26	71	pos	-	+	0	Duodenal ulcer	
7 H A	2-9-26	47	pos	-	+	0	Diabetes and cholelithiasis	
7 M B	2-23-26	83	pos	-	+	0	Diabetes and cholelithiasis	
8 M R	2-19-26	88	pos	-	+	0	Fernicious anemia	
9 C W	3-4-26	83	pos	-	+	0	Fernicious anemia	
10 E F	3-1-26	78	pos	-	+	0	Ranti s disease	
11 M W	1-29-26	100	pos	-	+	0	After cholecystectomy	
12 S D	4-9-26	100	pos.	-	+	0	Chronic cardiovascular disease	
13 J A	4-8-26	115	pos	-	+	0	Bronchopneumonia	
14 S R	4-26-26	107	pos	-	+	0	Bronchopneumonia	
15 A S	9-10-26	136	pos	-	+	0	Tuberculosis	
16 E S	6-5-26	125	pos	-	+	0	Tuberculosis	
	6-6-26	115	pos	-	+	0	Acute cholecystitis	
	6-9-26	111	pos	-	+	0	Acute cholecystitis	
	6-11-26	100	pos	-	+	0	Acute cholecystitis	
17 W G	1-1-26	111	pos	-	+	0	Acute cholecystitis	
18 J T	4-24-26	16	pos	-	+	0	Acute cholecystitis	
19 J P	2-6-26	136	pos	-	+	0	Acute cholecystitis	
20 H Y	2-1-26	166	pos	-	+	0	Acute cholecystitis	
21 A M	2-1-26	166	pos	-	+	0	Acute cholecystitis	
22 J B	5-10-26	222	pos	+	+	+	Splenomegaly and 2ry anemia	
23 M W	1-8-26	50	0	-	+	+	Von Jaeksch anemia	
24 O C	1-8-26	62	06	-	+	+	Laetic hepatitis	
25 I H	6-3-26	88	10	-	+	+	Angina pectoris	
26 I H	1-8-26	93	06	-	+	+	Angina pectoris	
27 I W	1-11-26	107	11	-	+	+	Angina pectoris	
28 A S	7-9-26	100	28	-	+	+	Angina pectoris	
29 I S	3-1-26	125	14	-	+	+	Angina pectoris	
30 R O	2-4-26	136	25	-	+	+	Cholecystitis	
31 B P	4-21-26	107	11	-	+	+	Fernicious anemia	
							Duodenal ulcer	
							Chronic cardiovascular disease	
							Cardiac decom	

TABLE I—CONT'D

PATIENT	DATE	ICTERUS INDEX	AZOBILIRUBIN MG PER 100 CC		VAN DEN BERGH		CLINICAL JAUNDICE	DIAGNOSIS
			DIRECT	INDIRECT				
35 M G	8-16-26	125	18	+	—	+	±	Permeous anemia
32 J P	8-23-26	107	21	+	—	+	0	Chronic myocanditis
37 A T	1-8-26	136	29	+	—	+	0	Carcinoma of breast
34 E C	2-15-26	125	15	+	—	+	0	Chronic cardiovascular disease
29 S B	2-19-26	100	11	+	—	+	0	
39 B B	2-8-26	150	22	+	—	+	+	Catarrhal jaundice
	2-19-26	78	pos	+	—	+	+	
38 J T	2-3-26	130	17	+	—	+	+	Hodgkin's disease, enlarged liver
	2-5-26	107	15	+	—	+	±	
42 E K	4-22-26	204	21	+	+	+	+	Lobar pneumonia
43 M D	8-22-26	205	35	+	+	+	+	Typhoid fever, cholecystitis
41 J M	8-20-26	200	27	+	—	+	0	Chronic cholecystitis
44 E R	8-11-26	221	35	+	—	+	+	Permeous anemia
45 M B	5-17-26	230	40	+	+	+	+	Secondary malignancy of liver, primary not known
	5-20-26	204	25	+	+	+	+	
	5-28-26	204	25	+	+	+	+	
46 I S	6-28-26	249	34	+	—	+	+	Abdominal adhesions right upper quadrant
	6-30-26	176	24	+	—	+	+	
47 F B	2-26-26	267	42	+	—	+	+	Cholecystitis with adhesions
40 E C	1-20-26	187	18	+	—	+	++	Stricture of common duct after cholecystectomy
	2-3-26	440	56	+	—	+	+	
	6-5-26	110	pos	+	—	+	0	
	6-7-26	110	pos	+	—	+	0	
	6-11-26	180	20	+	—	+	+	
48 A D	4-21-26	321	30	+	+	+	+	Postoperative sepsis Hemolytic streptococcus
49 S E	12-29-26	357	34	++	+	+	++	Purpura hemorrhagica
	1-8-26	150	15	+	+	+	+	
50 J S	3-18-26	408	44	+	+	+	+	Acute cholecystitis and cholelithiasis
51 C P	3-25-26	408	59	++	+	+	++	Splenomegaly Cholelithiasis
	3-27-26	400	69	++	+	+	++	
52 J S	3-18-26	410	44	+	+	+	++	Chronic cholecystitis
53 M C	8-17-26	428	56	++	+	+	++	Catarrhal jaundice
	8-23-26	333	73	++	+	+	++	
	1-11-26	465	39	++	+	+	++	Cholecystitis and cholelithiasis
54 M L	2-24-26	372	39	++	+	+	++	
	3-5-26	332	39	++	+	+	++	

TABLE I—CONT'D

PATIENT	DATE	ICTERUS INDEX	AZOBILIRUBIN MG PER 100 CG	VAN DEN BEECH		CLINICAL JAUNDICE	DIAGNOSIS
				DIRECT	INDIRECT		
55 E K	1-8-26	500	71	+++	++++	++	Carcinoma of biliary tract, metastasis to liver
	1-13-26	996	124	++++	++++		
	1-28-26	900	124	++++	++++		
	2-11-26	1300	131	++++	++++		
56 C L	2-26-26	600	95	++++	++++	++	Lytic hepatitis
	3-10-26	750	74	++++	++++		
57 A. M	4-9-26	720	93	++++	++++	++	Cholelithiasis
	4-17-26	375	36	++++	++++		Postoperative
58 G L	5-11-26	825	100	++++	++++	+++	Malignancy obstructing common duct
	5-21-26	817	92	++++	++++		
59 B F	6-16-26	867	158	++++	++++	+++	Cirrhosis of liver
60 M S	4-3-26	916	101	++++	++++	++	Cholelithiasis
61 R S	7-12-26	923	93	++++	++++	++	Malignancy of biliary tract
	7-16-26	1071	129	++++	++++		
62 J B	6-3-26	950	117	+++	+++	+++	Carcinoma of pylorus common duct
	4-28-26	1050	173	+++	+++		
63 U M	5-3-26	1420	191	+++	+++	++	Catarrhal jaundice
	5-10-26	500	74	+	+++		
	5-14-26	650	88	+	+++		
64 S M	3-13-26	1100	138	++++	++++	+++	Malignancy of biliary tract
	3-20-26	1490	172	++++	++++		
65 V M	7-26-26	1120	140	++++	++++	+++	Carcinoma of stomach plate obstruction
	6-21-26	1360	202	++++	++++	+++	Metastasis to liver
66 J C	7-3-26	600	83	++++	++++	+++	Carcinoma of pancreas
	3-24-26	1500	210	++++	++++	++	After cholecystogastrostomy
67 M R	3-30-26	2250	310	++++	++++	+++	Malignancy obstructing common duct
	4-5-26	1600	250	++++	++++		
	4-16-26	2160	270	++++	++++		
68 E B	8-11-26	1660	296	++++	++++	+++	Chronic cholangitis
	8-12-26	2020	304	++++	++++		Structure of common duct
	5-18-26	1420	132	++++	++++		
69 V S	2-27-26	1875	249	++++	++++	+++	Icterus gravis neonatorum

Since the appearance of this paper by Greene, Snell and Walters we have included in our studies of the bilirubinemia in cases of jaundice or with involvement of hepatic function, then modification of the van den Bergh method. In this communication we are presenting the results obtained for the icterus index, the qualitative and quantitative van den Bergh tests. The object of this study was to determine whether the results obtained by this more complicated van den Bergh technic warranted its adoption as a standard laboratory method for bilirubinemia in place of the icterus index. Parallel determinations of the bilirubin of the blood serum by these two methods have been made in 150 cases representing a wide range of hyperbilirubinemia. Of these 69 representative cases are reported in the table. All of the cases, except three (11, 14 and 54) were hospital patients. The blood was obtained after a night's fast and after a carotin-free diet for twenty-four hours. In the majority of instances the disc was used as a standard for the icterus index. For the ferric thiocyanate standard of the van den Bergh method, Merck's reagent quality ferric ammonium sulphate was recrystallized and dried in a desiccator over calcium chloride. The qualitative van den Bergh tests were separate procedures as described by McNee,¹⁰ and not based upon color changes in the quantitative test as proposed by Greene, Snell and Walters.

In Table I are presented the findings for 69 cases, arranged in the order of their icterus indices. Of these 45 individuals had icterus indices varying from 3.3 to 24.9. The first 23 cases reported show indices from 3.3 to 22.5, but in these instances a satisfactory determination of the serum bilirubin could not be obtained by the quantitative van den Bergh method as described by Greene, Snell and Walters. In the first case no color was produced in the diazo reaction, but in the remaining tests the shade of color of the unknown varied to such a degree from the standard that a comparison in the colorimeter was impossible. In these instances the quantitative van den Bergh reaction is reported as positive in Table I, but an accurate estimate of the serum bilirubin could not be made. It is evident from an inspection of the table that 19 of these cases were within the zone of latent jaundice. It is particularly for cases in this stage of jaundice that a knowledge of the serum bilirubin is most essential. In the following 22 cases having icterus indices from 5.0 to 24.9 the color comparisons in the quantitative van den Bergh method were sufficiently satisfactory to warrant the calculation of azobilirubin in terms of mg per 100 cc of serum. In many of these instances the color shades varied, but the readings could be confined to a small range on the colorimeter scale. The azobilirubin concentration in these sera varied from 0.5 to 4.0 mg per 100 cc. It is seen, however, that the figures for the icterus index and the azobilirubin do not run parallel. In Case 30, an icterus index of 10.7 was obtained with an azobilirubin of 1.1 mg per 100 cc, but in Case 28 the icterus index was but 10.0 and the azobilirubin 2.8 mg per 100 cc. In Case 39, when the icterus index was 15.0, the azobilirubin was found to be 2.2 mg, but eleven days later the icterus index had dropped to 7.8, and an accurate determination of the azobilirubin could not be made. Again in Case 40, when the serum bilirubin drops to within the limits of latent jaundice the azobilirubin determination becomes unsatisfactory.

A satisfactory color comparison of the unknown with the standard was made in all specimens of blood serum with icterus indices exceeding 25. However, in these cases the determinations of the azobilirubin do not parallel the icterus indices in changes in the bilirubin content of the blood serum. In the data reported by Snell, Greene and Rountree¹ on these tests for hepatic function in experimental obstructive jaundice, it is also seen that their figures for serum bilirubin by their quantitative technique do not parallel the "bile indices." The normal serum bilirubin content according to Greene, Snell and Walters is from 0.3 to 1.4 mg per 100 cc. In many instances in the cases studied by us (Cases 26, 27, 29, 30, 31, 33) normal figures were obtained for the serum bilirubin, the icterus indices however, were definitely increased above normal. The increased icterus indices were accompanied by positive indirect van den Bergh reactions. Moreover in other instances with similar icterus indices abnormal figures for the serum bilirubin were found. It is evident from the dates of analyses reported in the table that the unsatisfactory determinations of the serum bilirubin cannot be attributed to a lack of experience with the method. The chemical technique was beyond reproach and the chemicals utilized were of the highest obtainable grade of purity. It is in those cases where the serum bilirubin lies between the range of normal concentration and frank clinical jaundice that the results of the quantitative van den Bergh method are least dependable. Bernheim also reports in her experience that the zone of latent jaundice cannot be accurately defined by this procedure.

We believe the sources of error are inherent in the method itself. At the present time it is not possible to check the standard against pure bilirubin. This fact alone opens the method to criticism and the results obtained are of comparative value only. The figures no more represent the actual amount of bilirubin in the blood serum than does the icterus index. Ether is not an ideal solvent for the standard particularly when a less volatile solvent alcohol, is used for the unknown. Owing to the evaporation of ether in the colorimeter cup the standard can be used for but one comparison. Moreover it is difficult to determine precisely when the coupling of the bilirubin with the diazonium solution has been completed. It was believed by the sponsors of this method that its use would obviate errors due to carotinemias and lipemias but in our experience the occurrence of carotin or lipins in the blood serum influences the shade of color in the diazo reaction so that an accurate determination of the bilirubin cannot be made.

SUMMARY

In 150 cases representing varying degrees of jaundice parallel determinations of the serum bilirubin were made by the icterus index of the blood serum and the quantitative van den Bergh method as modified by Greene, Snell and Walters. When precautions were taken to avoid errors due to hemolysis, lipemia and carotinemias, the determination of the icterus index was found to be the more reliable measure of the serum bilirubin. The estimation of the serum bilirubin by the quantitative van den Bergh technique was unsatisfactory in about 50 per cent of the cases within the zone of latent jaundice. The

simplicity of the method recommends the icterus index as a standard laboratory procedure. The quantitative van den Bergh method cannot be standardized, and the technic is subject to many sources of error.

REFERENCES

- ¹Blankenhorn, M. A. Arch Int Med, 1917, xix, 344
- ²Meulengracht, E. Deutsch Arch f klin Med (Leipzig), 1921, cxxvii, 38
- ³Stetten, D. Ann Surg, 1922, lxxvi, 191
- ⁴Bernheim, A. Jour Am Med Assn, 1924, lxxxii, 291
- ⁵Meulengracht, E. Ibid
- ⁶Hess, A. F., and Myers, V. C. Jour Am Med Assn, 1919, lxxiii, 1743
- ⁷Greene, C. H., Snell, A. M., and Walters, W. Arch Int Med, 1925, xxxvi, 248
- ⁸Bernheim, A. Arch Pathol and Lab Med, 1925, i, 747
- ⁹Preston, M. Jour Lab and Clin Med, 1926, vi, 879
- ¹⁰Barrow, J. V., Armstrong, E. L., and Olds, W. H. Am Jour Med Sci, 1925, clxx, 583
- ¹¹Shattuck, H. F., Browne, J. C., and Preston, M. Am Jour Med Sci, 1925, clxx, 510
- ¹²Schamberg, J. F., and Brown, H. Jour Am Med Assn, 1924, lxxxii, 1911
- ¹³Thannhauser, J. S., and Andersen, E. Deutsch Arch f klin Med, 1921, cxxviii, 179
- ¹⁴Blankenhorn, M. A. Arch Int Med, 1922, xxvii, 131
- ¹⁵Thannhauser, J. S., and Andersen, E. Ibid
- ¹⁶McNee, J. W. Quart Jour Med, 1923, vi, 390
- ¹⁷Snell, A. M., Greene, C. H., and Rountree, L. G. Arch Int Med, 1925, xxxvi, 273

THE PREPARATION OF COLLOIDAL GOLD SOLUTION*

By ROBERT A. KILDUFFE, M.D., AND W. W. HERSOHN, ATLANTIC CITY

THE multiplicity of methods which have been described for the preparation of colloidal gold solution is, in itself, an indication that no one method is entirely satisfactory nor certain to produce good solutions with unvarying certainty.

The preparation of the solution, therefore, still remains the only part of the test presenting any difficulty.

Certain facts are clear from the many studies which have been made. It is evident, for example, that the laborious and time-consuming procedure originally considered essential by the earlier investigators is not only not necessary but even this, despite its careful precautions throughout every step, cannot always be relied upon to produce a satisfactory solution.

It is also known that good solutions must be absolutely neutral, acid solutions being too sensitive and alkaline solutions too insensitive to conform to the standards required, namely, no reaction with a normal spinal fluid, and complete reduction of 5 c.c. of solution by 17 c.c. of 1 per cent sodium chloride solution within one hour.

Undoubtedly, the predominant factor in producing unsatisfactory solutions is a lack of neutrality in the finished product which, regardless of the method used, may be due to a variety of causes such as the water used or varying degrees of acidity or alkalinity in the various reagents.

*From the Laboratories of The Atlantic City Hospital Atlantic City N. J.
Received for publication Nov. 4, 1926.

Many methods have been introduced to overcome this difficulty either during the preparation of the solution or by various measures after its completion

A simple test of the reaction by means of alizarin as an indicator as commonly advocated is unsatisfactory because of the difficulty in reading the color change and it is more or less a common experience that solutions requiring extensive adjustment are apt to be of mediocre value

For this reason other methods have achieved some vogue such as that described by Mellanby and Anwyl Davies¹ the modification of it described by Haden, and that described by Novick² the two former aiming to secure neutrality before, and the latter after the completion of the solution

In our experience the Mellanby Anwyl Davies technique has always produced slightly acid and, hence, too sensitive solutions and the Haden modification solutions tending toward a slightly alkaline reaction

Novick's titration measurement of the reaction is quite satisfactory but, while indicating the correction needed does not obviate the necessity for, at times, marked readjustment of the solution tested

It occurred to us, therefore to combine the good features of all methods by which means the uniform and invariable preparation of good solutions is certain and a matter of relative simplicity

The entire procedure follows

Glassware Must be free from scratches and thoroughly cleaned We use Pyrex flasks which are cleaned in sulphuric acid dichromate mixture followed by prolonged rinsing in flowing tap water and finally in numerous changes of distilled water Test tubes are similarly treated

Reagents All solutions are made with distilled water Those required are

N/20 Hydrochloric acid

N/20 Potassium hydroxide

One per cent gold chloride solution Gold chloride Merek, 15 grains distilled water 100 c c

One per cent potassium oxalate Potassium oxalate, C P neutral, 1 gram, distilled water 100 c c

One per cent potassium hydroxide Potassium hydroxide C P purified by alcohol, 1 gm in 100 c c distilled water

These solutions are stable

Distilled Water Double distillation is necessary the second distillation being just prior to use As noted by Haden it is an advantage to add 1 c c of 10 per cent potassium permanganate and 1 c c of a saturated solution of barium hydroxide to each 2 liters of water just before the second distillation

Preliminary Titration

To each of six clean test tubes in a rack add 1 c c of 1 per cent gold chloride and add the following amounts of 1 per cent potassium hydroxide beginning with tube 1 and ending with tube 6 0.6 c c, 0.5 c c, 0.4 c c, 0.3 c c, 0.2 c c, and 0.1 c c

A varying degree of turbidity will be noted in accordance with the degree of alkalinity, the most alkaline tube remaining clear

Now add to each tube 1 c.c. of 1 per cent neutral potassium oxalate. Reduction begins at once. Complete reduction is indicated by a dense black precipitate, partial reduction by a lead color.

The tube containing the largest amount of alkali which can be added is that which shows complete reduction, usually tube 4 containing 0.3 c.c. of alkali.

Preparation of Solution

To 100 c.c. of double distilled water in a Pyrex flask add 1 c.c. of 1 per cent neutral potassium oxalate and heat to boiling.

While this is heating, in a clean test tube place 1 c.c. of 1 per cent gold chloride and add the amount of 1 per cent potassium hydroxide solution indicated by the preliminary titration.

When the contents of the flask are boiling run in the gold chloride alkali mixture drop by drop. The clear, red color develops at once when the solution is removed from the flame and allowed to cool.

The only objection to the method lies in the fact that it does not seem possible to prepare the solution in quantities greater than 100 c.c. at a time. As any number of solutions may be prepared quickly, added together, and corrected at once, as noted below, this objection is slight.

Final Titration and Correction

When a sufficient volume of solution has been prepared, mixed, and allowed to cool, place 5 c.c. in a clean tube and add 1.7 c.c. of 1 per cent sodium chloride solution and set aside for one hour at room temperature.

If the solution is neutral and requires no correction, complete precipitation occurs rapidly, if alkaline, reduction is incomplete or absent in one hour.

Having thus determined in which direction correction is required, place eleven tubes in a rack and in each place 5 c.c. of the colloidal gold solution.

Now add, beginning with tube 1 and proceeding to tube 10, the following amounts of N/20 acid or alkali (as indicated by the saline tube just described): 0.05 c.c., 0.075 c.c., 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.25 c.c., 0.275 c.c., 0.3 c.c., 0.35 c.c., 0.375 c.c., and 0.4 c.c.

The eleventh tube is the control and receives no acid (or alkali).

Now add to all tubes 1.7 c.c. of 1 per cent sodium chloride and set aside for one hour at room temperature protected from light.

The tube showing complete precipitation and containing the least amount of acid (or alkali) shows the measure of correction required for 5 c.c. of solution from which the amount needed for the total volume of solution at hand is readily calculated.

(Acid or alkali solutions stronger than N/20 cannot be used as they affect the strength of the sodium chloride solution used as indicator.)

With the method outlined solutions can be prepared requiring a minimum adjustment of reaction and which will invariably give normal curves with normal fluids and parietic curves with parietic fluids—a sine qua non before any colloidal gold solution is put into use.

REFERENCES

- ¹Mellanby, J, and Anwyl Davies T The Precipitation of Colloidal Gold Solution by Cerebrospinal Fluid Brit. Jour Exper Path 1923 iv, 132
 Haden, R L A Note on the Preparation of Colloidal Gold Solution by the Mellanby Anwyl Davies Technic, JOUR. LAB AND CLIN MED Jan 1925, x, 4, 310
²Novick N The Correction of Colloidal Gold Solutions as Applied to the Lange Reaction Arch Neurol and Psych, April 1926, xv, 4 471

A MODIFICATION OF THE ICTERUS INDEX*

By REED ROCKWOOD M D, AND ADAM SZCZYPINSKI, BALTIMORE MD

IN THE method commonly used for determining the color of the serum, the serum either diluted or undiluted is matched against a standard solution of potassium dichromate or a glass standard. The resulting comparison is known as the icterus index. With the standard of 1 to 10,000 dichromate, which is used at present, normal blood with the colorimeter set at fifteen gives readings around three or four. This setting of the standard also gives a color which is rather pale for accurate matching. It is a well known fact that the errors inherent in colorimetric work are accentuated when the two columns of fluid are not approximately of the same length.

It has seemed to us desirable then, to modify the standard and setting so as to increase the strength of the color and to bring the columns to nearly equal lengths when used with normal bloods. Some of the standard can be poured into a graduated cylinder and the serum diluted to an approximate match in a similar cylinder. The standard can then be poured into the colorimeter cup and the factor of blood dilution taken care of in the calculation. This preliminary procedure is only necessary when the quantity of pigment is high.

We now make a solution of 3 to 10 000 dichromate (300 mg per liter of water). This is preserved with a few drops of concentrated sulphuric acid in a dark bottle. The standard solution is set at 20 in the colorimeter and the reading made accordingly. To bring the results back into the terms now reported in the literature for the icterus index a slightly modified calculation is used which is given below

$$\frac{20 \times 3 \times \text{number of dilutions}}{\text{Reading of unknown}} = \text{Icterus index}$$

From the Department of Medicine University of Maryland
 Received for publication October 25 1926

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A KILDUFFE, M D , ABSTRACT EDITOR

PREGNANCY Lactic Acid Content of the Blood During Pregnancy, Schultze, K. F
Zentralbl f Gynak, July 3, 1926, 1, 1759

The average normal value was found to be 11 mg per cent in nonpregnant women

In thirty seven pregnant women the average lactic acid content was 15 mg per cent
There was some fluctuation The author believes that lactic acid increases in the blood in
the later months of pregnancy for reasons not clear

In two cases of eclampsia the lactic acid content was enormously increased

AGRANULOCYTOSIS The Question of Agranulocytosis, Feer, W Schweiz med Wchn
schr, June 5, 1926, lvi, 551

Feer contends that the condition first described in 1922 and since known as agranulo
cytosis is not an independent disease but a variety of septic disease representing an atypical
sepsis and should be called "sepsis agranulocytotica "

BLOOD PRESSURE Circulatory Tonics vs Circulatory Depressants, Andrews, C L
Jour Am Med Assn, Sept 18, 1926, lxxxvii, 928

Andrews calls attention to the fact that many physicians and still more patients are
focusing attention upon the blood pressure to the neglect of the underlying causative factors,
and emphasizes that if such cases are properly classified the blood pressure will take care of
itself He emphasizes that

There is abundant evidence that many treat hypertension as a disease itself

There is a widespread fear that digitalis raises the blood pressure and should not be
used in hypertension

These cases can be partially classified by watching the blood pressure in conjunction
with treatment results

Tonic doses of digitalis should be given in hypertensive cases of long standing to sup
port the heart muscle

Patients with hypertension of long standing do better if the blood pressure is not low
ered too much

HYPERTENSION Ultimate Results of Essential Hypertension, Paulin, J E Jour Am
Med Assn, Sept 18, 1925, lxxxvii, 925

In a review of seventy six cases of essential hypertension observed from five to seven
teen years, the number of cases was about equally divided between the two sexes

The mortality for the group of men was 48.7 per cent, and for the women, 9.2 per
cent, a difference in favor of the women of 39.5 per cent During the five to seven year
period of observation, the mortality was remarkably higher among the men

Myocardial failure occurred earlier than cerebral hemorrhage among the men It oc
curred much earlier in men than in women

Death from cerebral hemorrhage in a majority of the cases was preceded by a previous
apoplectic seizure The greater number of deaths occur because of heart and blood vessel
weakness

The renal involvement in the late stages of this disease is usually very slight, only one
death occurring in the series because of renal failure

Essential hypertension occurring in women about the time of the menopause is rela
tively benign and is associated with fewer accidents and complications than for a similar
group in men.

No definite conclusions can be drawn as to the end result in a given case from a study of the blood pressure alone

No definite prognosis can be given from a study of the blood pressure the prognosis depending on the integrity of the heart and blood vessels

NEPHRITIS Experimental Production of Acute Glomerulonephritis Use of Active Principle of Scarletinal Streptococcus and a Consideration of Chronic Interstitial Changes Preliminary Report Duval O W and Hibbard R J Jour Am Med Assn, Sept 18, 1926 lxxxvii 898

The various types of acute glomerulonephritis including the epithelial "crescent" endothelial proliferation, hyaline thrombi in the vessels of the glomeruli hemorrhage into the capsular space and complete necrosis of capillary tufts can be produced experimentally in the rabbit under preserved conditions with the toxic principle of the scarlatinal streptococcus of the Dicks

The experimental production of the nephritis here reported is of unusual significance since it affords the opportunity to study the acute lesions in the order of their related sequence, and may form a means of tracing the changes that lead to a progressive diffuse nephritis Furthermore it opens a field of investigation that may lead to our better understanding of the causes and mode of production of certain forms of renal disease in man

Streptococcal nephritis induced in the rabbit and its complete analogy to the nephritic lesions of human scarlatina is of especial interest since the causal excitant is in keeping with injurious substances more likely responsible for nephritis in man. While experimental nephritis has been produced with substances such as cantharides, snake venom and urinum, these are improbably excitants of the human disease and for this reason have not afforded an accurate basis for comparative study

NEPHRITIS Thyroid Therapy and Thyroid Tolerance in Chronic Nephritis Epstein, A. A. Jour Am Med Assn, Sept 18, 1926 lxxxvii 913

The term chronic nephrosis is used to designate a group of cases in which a profound metabolic disturbance exists To this disturbance the name "diabetes albuminuricus" has been applied

The pathologic changes in the kidneys (tubular degeneration) are the consequence and not the cause of the metabolic disturbance

The therapeutic requirements in chronic nephrosis are met in some cases by high protein feeding alone and in others in conjunction with thyroid or thyroxin

Cases of chronic nephrosis exhibit an unusual tolerance for thyroid and thyroxin.

The response to thyroid therapy is best measured by the cholesterol content of the blood Thyrotoxic symptoms do not occur as long as a hypercholesterolemia exists

Certain cases of chronic nephrosis are susceptible of complete cure by the intelligent and persistent use of high protein feeding and thyroid therapy This may require a year or longer to accomplish

MENINGITIS Significant Chemical Changes in the Spinal Fluid in Meningitis with Special Reference to Lactic Acid Content, Osnato M. and Killman J A. Arch Neurol and Psychiatry Juno, 1926 xv 738

The lactic acid content of normal spinal fluid during the fasting and resting state varies from 6 to 10 mg per hundred c.c The lactic acid concentration of the spinal fluid bears a close relation to its concentration in the blood An increase of the lactic acid of the blood is associated with a similar increase in the spinal fluid and the reverse of this appears true

An increase of the lactic acid of the spinal fluid and of the blood was found in nephritis and epilepsy following the convulsions In epilepsy the spinal fluid after the convulsions gave figures for lactic acid exceeding those for the blood obtained at the same time.

Spinal fluids obtained from cases of meningitis showed high figures for lactic acid. The source of this increased formation of the lactic acid appears to be the cellular metabolism. In some instances no decrease was noted in the sugar in fluids in which the lactic acid was increased above normal, in others, however, no reaction for sugar was obtained. In no instance did the increase in lactic acid account for all the sugar lost.

SCARLET FEVER The Control of Scarlet Fever in Institutions, Colby, W. Jour Am Med Assn, Sept 18, 1926, LXXVII, 919

Positive Dick reactors can be immunized against scarlet fever streptococcus toxin. Children under eight years of age may be safely given 3,000 skin test dose with this method.

In young children immunity is effected within as short a period as eight days, which makes possible the suppression of an epidemic by active immunization.

In older children, while immunity is established more slowly, repeated Dick tests at three months and six months indicate a marked progressive immunization.

On observing the possibility of mild scarlet fever developing in negative Dick reactors, it becomes evident that the strength of the test material should be increased.

PREGNANCY Blood Pressure and Urinary Findings in 100 Cases of Normal Pregnancies, Faught, F. A. Jour Obst and Gynec, May, 1926, LI, 5

There is practically no difference in the average blood pressure values in the primipara, as compared with the multipara.

There is slightly greater tendency for primiparae to show urinary abnormalities, which, however, does not appear to have any great significance.

We may expect to find a high incidence of albumin in the urine of pregnant patients, associated in many instances with cases and red blood cells.

The influence of these abnormalities on the average blood pressure findings is insignificant and well within recognized normal variations.

The persistent occurrence of albumin and other urinary abnormalities usually has little significance.

Individuals, not infrequently, show marked abnormal variations in systolic pressure, both below and above the normal limits, the occurrence of which does not necessarily indicate impending grave metabolic disturbances or toxic states.

The occurrence of glucose and indican as complicating factors during pregnancy must be taken into consideration since their incidence is comparatively frequent, and may be associated with comparatively great blood pressure abnormalities in the individual case.

Their significance and effect upon the pregnant woman is probably no greater than the other urinary abnormalities.

The mere elevation of systolic blood pressure does not indicate the approach of grave complications unless persistent, under which condition further light should be sought by a study of the blood for the detection of nitrogen retention and disturbance in the CO₂ combining power of the blood.

PREGNANCY The Tendency to Acidosis in the Toxemia of Pregnancy, Levy, W. E. Surg, Gynec and Obst, July, 1926, 38

Eclampsia and preeclamptic toxemia are diseases of pregnancy manifested primarily by destruction of liver tissue. The liver is concerned with carbohydrate metabolism and storage. A deficiency of carbohydrates in the body leads to an imperfect combustion of the fats and in turn to the production of acetone bodies.

The author believes that he has definitely established that in the preeclamptic state and in eclampsia, an acidosis exists. Believing also that a distinct difference exists between preeclamptic toxemia and a toxemia due to a previous kidney disease, he has attempted to classify his cases as such.

Briefly he has included among those cases of preeclamptic toxemias such as show practically no renal involvement.

With the cases thus divided, the blood of the two types was next investigated along with the normal controls. As a result of the examination of the blood of fifty pre eclamptics and eclamptics it was found that there was a marked decrease in the blood sugar content, a lowering of the carbon dioxide combining power, and practically no change in the nitrogenous constituents.

He concludes that the toxin of eclampsia produces definite destruction of the liver lobules.

The destruction of liver substances causes a derangement of the carbohydrate metabolism and glycogen storage.

The blood sugar and carbon dioxide combining power are lowered.

A state of acidosis is either imminent or present.

The rational treatment is with glucose or glucosio and insulin.

He concludes that the outstanding chemical findings in the blood of eclamptic patients are:

- 1 A high uric acid
- 2 A markedly increased lactic acid not wholly due to muscular hyperactivity
- 3 A decrease in the CO₂ combining power which is very pronounced in certain cases.
- 4 A definite tendency towards a hyperglycemia which is often associated with a high inorganic phosphorus.

There is usually no increase in the nonprotein nitrogen of the eclamptic blood. When such an increase is present it may be associated either with a nephritis upon which the eclampsia has been superimposed or with the last stages of the disease. Furthermore, there is a slight, but definite decrease in the blood urea nitrogen as has already been pointed out by one of us.

It cannot be stated at present in how far the eclamptic blood picture is dependent upon the lesions usually observed in the liver.

TUBERCULIN New Method for Tuberculin Test. A New Method of Dermo Reaction and Its Clinical Value in the Examination of Adults. Ferrari A. *Rev. Med. Chir., Brazil*, November, 1925 LXIII, 627.

Ferrari makes an intracutaneous puncture and introduces tuberculin with a fine probe. The excess is removed after two minutes and the patient dismissed for five days.

When positive the reaction shows a reddish spot distinctly visible which may increase for several days, after which there is a slight desquamation and pruritus and finally a slight pigmentation lasting several weeks.

POLIOMYELITIS An Outbreak of Poliomyelitis Apparently Milk Borne. Knapp A. C., Godfrey, E. S. and Aycock W. L. *Jour. Am. Med. Assn.* April 28, 1926 LXXXVII, 635.

Detailed epidemiologic study apparently demonstrating that this disease can be spread by milk.

PERNICIOUS ANEMIA The Common Picture of Sprue, Pernicious Anemia, and Combined Degeneration. Reed A. C. and Wyckoff H. A. *Am. Jour. Trop. Med.* May 1926, VI, No. 3, p. 221.

The authors believe that a common clinical entity is embraced by the accepted diagnoses of tropical sprue, pernicious anemia, and subacute combined degeneration of the spinal cord. Review of published case records of these diseases and a study of our own cases point strongly to their being different intensities of manifestation of a common toxin. This toxin seems to attack the three systems, i.e. the blood, the gastrointestinal tract and the cord, to varying degrees although nearly always in cases fully studied all three systems afford evidence of damage under any one of the three clinical diagnoses. For example, typical sprue may show evidence of cord changes and pernicious features in the blood. Typical Addisonian anemia shows evidence of sprue-like gastrointestinal changes and of cord degeneration. And finally, subacute combined degeneration of the cord is always associated with a progressive anemia which tends to become pernicious in character, and frequently with

achylia and other gastroenteric lesions. We suggest further that the toxin is more likely a group or type toxin than a unit chemical substance, and that its place of origin is in the digestive canal. Such a conception of these large disease groups means that sprue and pernicious anemia cannot be considered as unit diseases with a constant classical and characteristic type. But each is a group of variable clinical syndromes just as is the case in the group of what is called beriberi. This holds true to a lesser degree for combined degeneration.

Case reports are analyzed in the light of this conception.

POLIOMYELITIS A Skin Reaction in Poliomyelitis, Rosenow, E C. Jour Infect Dis, June, 1926, *LXXVIII*, No 6, p 529

Rosenow found that freshly isolated strains of the pleomorphic streptococcus, which produced flaccid paralysis in rabbits, when grown eighteen to twenty four hours in pancreatic digest heart muscle broth to which one part in ten of ascites fluid was added, and the culture killed with phenol, 0.5 per cent, or cresol, 0.3 per cent, yielded a useful toxic antigen.

The absence of marked reactions in persons fully recovered from poliomyelitis and who are known to be immune, the incidence of positive reactions inversely according to age, corresponding in general to the age incidence of poliomyelitis, the strongly positive reactions during the acute stage of the disease, and the negative reaction during convalescence, are considered as presumptive evidence that the test is a measure of susceptibility to poliomyelitis.

Numerous questions regarding the nature of the reaction have not yet been worked out. The immune serum prepared from horses with the pleomorphic streptococcus, and used with apparent benefit in the treatment of the early stages of poliomyelitis, has, however, a marked neutralizing power over the toxin, as determined by the skin reaction.

BACTERIOPHAGE Bacteriophagy in Urinary Infection. Part I. The Incidence of Bacteriophage and of *Bacillus Coli* Susceptible to Dissolution by the Bacteriophage in Urines. Presentation of Cases of Renal Infection in Which Bacteriophage Was Used Therapeutically, Larkum, W N. Jour Bacteriol, September, 1926, *LIII*, No 3, p 203

Routine studies of urines from patients having urinary infections revealed the fact that bacteriophage was present in about 25 per cent of the urines while *Bacillus coli* susceptible to the action of bacteriophage was present in the same proportion of the specimens. The urines in which the susceptible *colou bacilli* were found were not necessarily the same as those in which bacteriophage was demonstrated. Normal urines, that is, urines not known to contain bacteria, were found to be free of bacteriophage.

When individual cases rather than urines were considered it was found that over 36 per cent of the cases studied had bacteriophage in one or more of the specimens of urine examined, while over 40 per cent (not necessarily including the above 36 per cent), were infected with a *colon bacillus* capable of being dissolved by a race of bacteriophage.

Almost without exception, the chronic cases provided urines in which only resistant bacteria were found, while the acute were seldom due to this type of *colon bacillus*. Bacteriophage too was found exclusively in the urines from individuals having acute infections.

The incidence of bacteriophage and susceptible *colon bacilli* in males and females was affected by the above condition. In practically every instance the males were suffering with chronic infections. Consequently the males, except in one case, were never a source of bacteriophage or susceptible bacteria.

Four patients subjected to treatment with the bacteriophage showed definite improvement after the treatment.

Bacteriophage is not found in rabbits' urine when bacteria of any type except the lysogenic strains are put into the bladder and maintained there for varying periods of time.

The introduction of *colon bacilli* into the body by the enteral or intravenous route fails to cause bacteriophage to appear in the bladder.

Damage to the bladder wall by means of hydrochloric acid does not result in the appearance of bacteriophage in the urine

When introduced into the bladder, bacteriophage is eliminated within twenty four to forty eight hours.

As a result of these findings it is suggested that infection with lysogenic strains of *Bacillus coli* is alone responsible for the existence of bacteriophage in the urine

Lysis of colon bacilli through the action of the bacteriophage can take place in the bladder

Urine overcomes an inhibitive action upon bacteriophage

Mucus, although apparently not affecting bacteriophage, acts upon the colon bacilli in such a manner as to promote their removal from the bladder

Surviving bladder tissue has no effect upon bacteriophage

Dead bladder tissue releases a principle resembling bacteriophage

While it is impossible, on the basis of these experiments to state through what agency and to what extent modifications occur, it is obvious that bacteriophage is not the same in the bladder as it is in the test tube

PREGNANCY Interagglutination of Maternal and Fetal Blood in the Late Toxemias of Pregnancy Allen W M Bull Johns Hopkins Hosp 1926, xxxviii, 217

Allen investigated the iso agglutination characteristics of 375 normal and 104 toxemic women and their infants.

He found no evidence that the late toxemias of pregnancy originate in iso agglutination phenomena and believes previous reports based on too few cases

URIC ACID A Blood Uric Oxidase and the True Value of the Blood Uric Acid Flatow A. Munch med Wchnschr 1926, lxxiii, 12

Flatow believes that the blood uric acid is much higher normally than appears from present methods of determination, due to the error introduced by a uric oxidase derived from formed elements of the blood which is carried into the deproteinized filtrate, is active in weakly acid and alkaline solutions and is heat stable

EPILEPSY The Spinal Fluid in Epilepsy A Study of Fifty Cases Patterson H. and Levy P Arch Neurol and Psychiatry 1926 xv, 353

No significant changes were found other than a great increase in pressure during an attack and the relatively frequent occurrence of colloidal gold curves similar to those seen in cerebrospinal syphilis

NEOPLASMS Mitotic Figures in Malignant Tumors as Affected by Time Before Fixation of Tissues Evans N Arch Path and Lab Med June 1926 i, No 6 p 894

Evans has been accustomed to grade the malignancy of tumors in accordance with the number of mitotic figures present in sections which he reports in terms of the number per cubic millimeter of tissue

A study was made of two tumors to determine the effect keeping tissue unfixed for varying periods. No material variation was found in the number of mitotic figures present

PERNICIOUS ANEMIA Treatment of Pernicious Anemia by a Special Diet, Minot, G R. and Murphy W P Jour Am Med Assn, August 14, 1926 lxxxvii 470

The special diet used was made as palatable as possible and for each day was practically as follows

From 120 to 240 gm, and even sometimes more, of cooked calf's or beef liver. An equal quantity of lamb's kidneys was substituted occasionally

One hundred and twenty grams or more of beef or mutton muscle meat

Not less than 300 gm of vegetables containing from 1 to 10 per cent carbohydrate especially lettuce and spinach

From 250 to 500 gm of fruit, especially peaches, apricots, strawberries, pineapple, oranges and grapefruit

About 40 gm of fat derived from butter and cream, allowed in order to make the food attractive. Animal fats and oils, however, were excluded as far as possible

If desired, an egg and 240 gm of milk

In addition to the above mentioned foods, breads especially dry and crusty, potato, and cereals, in order to allow a total intake of between 2,000 and 3,000 calories composed usually of about 340 gm of carbohydrate, 135 gm of protein, and not more than 70 gm of fat. Grossly sweet foods were not given but sugar allowed very sparingly

This diet is rich in iron and purine derivatives containing about 0.03 gm of the former and about 1 gm of the latter

Forty five cases were thus treated with very encouraging results

GINGIVITIS The Chemotherapy of Gingivitis, Kolmer, J A Dental Cosmos, April, 1926

Kolmer emphasizes that no one organism or group of organisms can be regarded as the primary or secondary cause of gingivitis. Some cases are predominantly bacterial, in others spirochetal forms which may occur in approximately normal mouths are responsible, in still others of the "trench mouth" type the fusiform bacilli and spirochetes of Vincent are the etiologic agents of importance. The *E. buccalis* may have some secondary importance as carriers of organisms or as opening up pathways for them.

The important feature of treatment is the correct application of the medicament so as to secure intimate and frequent contact with infected tissues together with the least disturbance possible so as not to hinder healing processes or extend the process by trauma.

Surgical removal of necrotic tissues, etc., is necessary.

The following solution is very effective

Arsphenamine 0.3 gm
Hot water 15 c c
Dissolve and add 15 c c of glycerine

The solution is effective until oxidation has produced a blackish green color

A useful adjuvant is a tooth paste mercurochrome or metaphen 0.5 gm to 100 gm of tooth paste. This is rubbed into the gums with the finger and after a minute or two brushed off. A lotion may also be used several times a day. The following are suggested

Mercurochrome	0.1 gm
Peppermint water	100 c c
or	
Metaphen	0.1 gm
N/1 Sol NaOH	4 c c
Peppermint water	96 c c

INSULIN The Effect of Injections of Insulin and Dextrose on Blood Sugar, Thalheimer, W, Raine, F, Perry, M C, and Buttles, J Jour Am Med Assn, August 7, 1926, LVII, 391

The intravenous injection of 10 per cent dextrose at a slow rate into normal persons induces a more rapid removal of sugar from the blood, so that during the latter part of the injection the blood sugar level, instead of continuing to increase, actually declines.

Insulin mixed with the dextrose solution and given intravenously causes a more rapid and greater removal of sugar from the blood than when the insulin is given subcutaneously.

SMALLPOX Smallpox without Eruption Following Blood Stream Inoculation, Blalock, J R Annals Clin Med, March, 1926, IV, No 9, p 722

The day after a transfusion in a case of pernicious anemia the donor presented a smallpox eruption. Ten days later the recipient complained of headache and an erythematous rash appeared on the thirteenth day, the temperature never rising above 100° F.

The course of the disease was uneventful. Vaccination on admission and on the third day after transfusion was unsuccessful.

The author concludes:

Inoculation of the blood of a person within the incubation period and within the period of prodromal symptoms of smallpox into the blood stream of another individual produced within the recognized incubation period the prodromal manifestations of smallpox, including the prodromal rash.

The organism or infecting agent of smallpox is present in the blood stream at least twenty-four hours before eruption.

The clinical syndrome referred to as *variola sine eruptione* may be produced following blood stream inoculation in a person partially protected by vaccination.

A striking example is furnished of the protective value of vaccination.

VAGINAL FLORA The Vaginal Flora During Childhood and Puberty Soeken Gertrude. *Ztschr f Kinderh* Feb 20 1914 1177

In childhood the vaginal flora is predominantly a coccus flora but at the age of about eleven years this is in most cases replaced by a vaginal bacillus flora. This transformation is always connected with the presence of the signs of puberty. It takes place during an early stage of puberty often long before the first menstruation occurs and it is always a rapid and definitive change.

SCARLET FEVER The Preparation and Clinical Application of Scarlet Fever Antitoxin, Anderson, J F and Leonard G F. *Am Jour Med Sc* September 1926 clxxii No 6 p 634

A detailed and minute description of the methods used for the preparation of scarlet fever antitoxin. Because of its wealth of detail this paper cannot be abstracted satisfactorily short of transcription.

Analysis of the clinical results following the use of scarlet fever antitoxin prepared in a single laboratory in widely separated sections shows that the serum was specific for the various types of cases occurring in different sections of the United States.

The authors conclude that specific scarlet fever antitoxin may be prepared by the immunization of horses with filtered toxin.

Such antitoxin is specific against scarlet fever occurring in widely separated sections of the United States.

A properly prepared and standardized antitoxin is effective as a prophylactic when used in adequate doses.

When used for passive immunization it should be given in not less than one-half of the average therapeutic dose.

A properly prepared and standardized scarlet fever antitoxin is effective in the treatment of scarlet fever saving life and reducing the severity and frequency of complications.

GOITER Histologic Changes Following Administration of Iodine in Exophthalmic Goiter Giordano A S. *Arch Path and Lab Med* June 1926 1 No 6 p 331

From a study of glands taken at necropsy from exophthalmic goiter patients dying during crises there was found evidence that in most instances involution changes in the thyroid gland occur when iodine is administered to patients with exophthalmic goiter, and that, in general, the degree of involution of the thyroid parenchyma closely parallels the clinical course. The changes are similar in character to those described following ligation of the thyroid vessels but they occur rather uniformly throughout the gland. It seems fair to assume that these changes are not characteristic of the method that induces them for the author has also observed them in patients who came to operation during a period of remission of the clinical symptoms without any therapy other than rest. On the other hand we have yet to explain the occurrence of marked involution changes in patients with definitely active true ophthalmic goiter. Such an occurrence is admittedly rare but as yet no definite explanation has been given. This suggests that the anatomic picture does not always parallel the clinical course.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

Diseases of the Heart[†]

THE second and third editions of this work contained few alterations from the first. Indeed, there were five printings of the third edition between 1913 and 1921 with practically no revision. The fourth edition, however, which was in proof at the time of the author's death has been quite extensively rewritten and brought up to date. The volume is a vehicle for the exposition of Sir James Mackenzie's theories of the physiology and pathology of the heart. As he has done in many of his more recent contributions, he points out the chief errors of the present system of medical research and the lack of finality of conclusions reached thereby, and points and leads the way to deeper and more thorough studies of fundamental principles.

"The methods of investigation pursued hitherto have led to the accumulation of a mass of symptoms and reactions. Most investigators end by adding to this mass and by introducing some new term. So confused is this mass that it is beyond the comprehension of any individual, and one result is that it effectually obscures the path of progress, so that the investigator himself raises a barrier to further progress.

"As all investigators are practically dealing with the same phenomena, and as the investigator in each branch sees the phenomena under different circumstances, each one applies a name which meets his own notion. The result is that the worker in one field is unable to understand the language of workers in other fields, although they are all dealing with the same kind of phenomena. There is thus lost that community of ideas and coordinate participation in work which is so essential to progress. The question arises, how can medical investigation be carried beyond this stage? Manifestly by understanding the factors concerned in the production of the symptom or reaction."

No instrument for the measurement of the functional efficiency of the heart or circulation now known will prove to be satisfactory in the opinion of the author.

"Before we employ any test we must know the nature of our measure. The employment of a measure as a measure without knowing what it measures gives a useless kind of knowledge or leads to fallacious results. Because a foot rule can measure a yard of cloth, it does not follow that it can measure a pint of beer. Because an increased rate of pulse may indicate the sensitivity of the sinoauricular node, it does not follow that it can throw light upon the functional efficiency of the heart."

In his illustrations Mackenzie still uses polygraphic tracings in preference to electrocardiographic tracings because nearly as much information can be obtained therefrom and the investigator using the polygraph is studying natural phenomena which may later be studied without the aid of instruments, while the electrocardiologist is studying unknown forces. With the former the physician is studying movements which he can see and feel and must learn to recognize and to interpret. We know the forces that produce the various waves which are shown on a polygraphic record but it is not known what the agent is that produces the electro

[†]*Diseases of the Heart*. By Sir James Mackenzie FRS MD FRCP LL D
Ab & Ed FRCP (hon) Cloth Illustrated Pp 496 Humphrey Milford Oxford University Press

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

cardiogram. There is no sign of importance exhibited by the heart which cannot be recognized by the unaided senses. It behooves the student to familiarize himself with the knowledge to be gained from instruments of precision but chiefly for the purpose of improving his own ability to dispense with these instruments.

All of the various affections of the heart are discussed in detail and interpreted in the light of Dr. Mackenzie's theories.

This volume is Mackenzie's last word on diseases of the heart in general and should be in the library of every cardiologist.

*Goiter: Nonsurgical Types and Treatment*¹

THE author includes under this designation simple endemic goiter, adolescent hyperplasia and the like, and Graves' disease or exophthalmic goiter.

The major portion of the work is devoted to a consideration of exophthalmic goiter. Bram takes a most emphatic stand against the consideration or treatment of this disease as surgical. He takes the stand that the basic pathology is probably not primarily in the thyroid gland but consists of an endocrine imbalance together with a disturbance in the vegetative nervous system in which the thyroid is only incidentally playing a part. Whether this proposition be acceptable or not his further argument against surgery is obviously logical. If, as has been suggested by the workers at the Mayo Clinic, the symptoms of Graves' disease are brought about by an incompletely iodized thyroxin molecule, a thyroid dysfunction rather than hyperfunction, our endeavor should be to provide the requisite amount of iodine or otherwise establish the manufacture of a normal thyroxin rather than merely to cut down the supply of the abnormal secretion. This is the logic for the administration of Lugol's solution.

Subtotal thyroidectomy for Graves' disease will naturally cut down by the amount of thyroid tissue removed the amount of abnormal thyroxin manufactured and to this degree will relieve the symptoms of the disease but as the gland regenerates increasing amounts of the abnormal substance will again be manufactured and the symptoms will often return. The author insists that the high incidence of recurrences after operation is an argument against surgery. On the other hand he considers toxic adenoma surgical for in this condition the diseased tissue is encapsulated and may be removed in its entirety. In Graves' disease removal of the entire gland would eventuate in death from myxedema.

The author's treatment is nonsurgical and includes rest, psychotherapy, readjustment, iodine and quinine medication, etc., removal of infectious foci, hygienic measures and a high caloric low protein diet. X-ray or radium treatment is taboo as producing very much the same effects as surgery, namely partial destruction of the glandular tissue.

The author gives comparative statistics which would indicate better results from medical than from surgical treatment.

*The Surgery of Gastro Duodenal Ulceration*²

THE proper treatment of gastric and duodenal ulcer has long been a bone of contention between the surgeon and the gastroenterologist. Each claims superior results and presents statistics purported to demonstrate the inferiority of the medical and dietary treatment or the surgical end results as the case may be. The recent trend is more toward conservative methods but there are still too many surgeons who insist upon the necessity for operation immediately the diagnosis has been made.

In this book we find a surgeon who while writing on the surgical treatment of these diseases prefaces his dissertation with the statement that with few exceptions medical treatment should always be given thorough preliminary trial. He insists on the fact known to all but

¹Goiter: Nonsurgical Types and Treatment. By Israel Bram, M.D., Instructor in Clinical Medicine, Jefferson Medical College, Philadelphia, Pa. Cloth. Illustrated. Pp. 449. The Macmillan Company, 1924.

²The Surgery of Gastro-Duodenal Ulceration. By Charles A. Pannett, B.Sc., M.D. (Lond.), F.R.C.S. (Eng.), Professor of Surgery in the University of London, Surgeon to St. Mary's Hospital. Cloth. Illustrated. Pp. 154. Humphrey Milford, Oxford University Press.

some surgeons that ulcers undoubtedly heal under proper medical treatment, indeed not infrequently heal spontaneously with no treatment at all except that which nature imposes upon the sufferer by forcing him to rest and restrict his diet

Those conditions which are most likely to necessitate surgical intervention are hemorrhage, perforation, organic obstruction and large callous ulcers which, though they may heal, do so with such devitalized and poorly nourished tissue that they are continually breaking down with the formation of new ulcers

Dr Pannett presents a critical comparative analysis of the various operations recommended and designates when each should be used There are chapters devoted to perforation, hemorrhage, operative technic, and postoperative sequelae

*Greene's Medical Diagnosis**

WHEN a volume has passed through its sixth edition it may be said to have established its own value Greene's Medical Diagnosis is most ambitious in its scope and any who have been through it will agree that it approaches the realization of its ambitions Both in size and utility it is comparable to French's Index of Differential Diagnoses It differs from French in several respects, however French is purely a reference manual while Greene is a combination textbook and reference instrument Indeed, the volume may be best classified as a combined textbook on physical diagnosis and clinical pathology The section devoted to the heart and vascular system is exceptionally good Illustrations are bountiful throughout the book Roentgen interpretation is incorporated under the various subjects and roentgenograms are abundant A new section devoted to electrocardiography is profusely illustrated

Marginal notations on all pages enable the hurried reader to see at a glance the general content of paragraphs in the text and thus facilitates the more rapid finding of the particular subject for which one may be looking The index covers 160 pages and is most exhaustive This is essential in a work of this type and adds greatly to the value of the book

Nephritis†

THE difficulty of writing an authoritative treatise on nephritis which will still be up to date by the time it gets into print is obvious While there has been no great addition to or change in our understanding of the functional pathology of nephritis since the publication of Cushny's last monograph, short contributions of the highest merit are continually appearing which deal with very closely limited phases of the physiology or pathology of the kidney or related conditions

It is well that from time to time we should have a comprehensive review on the subject which will correlate the outstanding facts, monographs which in succession will mark periods of progress and will serve as stepping stones for those interested in the disease under consideration, and enable the reader to avoid the alternative of wading through an enormous volume of individual contributions

This function is satisfactorily fulfilled by Elwyn's volume on nephritis The author has little to say of essential hypertension, classifying these cases rather as renal arteriosclerosis He says that in these cases there is always a pronounced hyaline degeneration of the arterioles in the kidneys without similar changes elsewhere in the body

He proposes a new solution of the eclampsia question He discards the toxin theory and conceives of the process somewhat as follows "With the beginning of pregnancy and continuous through it there is a gradual increase in the irritability of the entire neuromuscular mechanism which has to do with the function of uterine contraction With the increase in the irritability of the neuromuscular mechanism, the rhythmic contractions of the uterus become stronger, finally terminating in the contractions of labor This increased irritability is prob

*Medical Diagnosis for the Student and Practitioner By Charles Lyman Greene M.D. Cloth Illustrated Pp 1468 P Blakiston's Son & Co Philadelphia Pa
†Nephritis By Herman Elwyn M.D. Assistant Visiting Physician Gouverneur Hospital New York N.Y. Cloth Pp 347 The Macmillan Company 1926

ably dependent upon a state of increased irritability of the presiding centers in the brain. The entire mechanism is placed on a higher plane of activity for the purpose of the final expulsion of the fetus.

"The close proximity of the centers for the vegetative functions in the brain permits the increased state of irritability to spread to the center for vasoconstriction in some cases. Impulses passing through the fibers of the thoracolumbar outflow then cause the whole neuromuscular mechanism of the arterial system to become more irritable, and the arterial vessels to be in a state of greater tonic contraction. The irritability of the entire neuromuscular apparatus for vasoconstriction increases with the increase in the irritability of the neuromuscular apparatus for uterine contraction in the course of pregnancy. It becomes more marked at the time of labor. When the irritability is sufficiently high it causes arterial spastic contraction to a varying degree, slowly or suddenly and initiates all the manifestations which we have considered the result of arterial spastic contraction."

*Diathermy with Special Reference to Pneumonia**

THIS is the second edition of a book previously reviewed in these columns. The author has added considerable material, particularly in the nature of case reports and has broadened the field of interest into more detailed consideration of the diathermy treatment of conditions other than pneumonia.

For those who are interested in the practical use of diathermy the chapters on Diathermy Technique will be helpful.

Hay Fever and Asthma A Handbook for the Patient†

TO JOSLIN in particular must go credit for the development of classwork with groups of sufferers from the same disease. After the diabetic instruction classes there came the nephritic classes and the asthma classes. Choudler Walker was as far as the reviewer knows, the first to institute class instruction in asthma. A natural sequence to this has been the development of textbooks or manuals for the instruction of the patient himself so that he may have a better understanding of the nature of his infirmity and be more competent to himself apply the remedial measures prescribed by his physician.

Dr. Balyeat has written a very readable handbook on hay fever and asthma. In no way does it supplant the physician himself. Certainly however the reader after digesting its contents should make a better patient, a more cooperative one and at the same time, a less impatient one.

The rationale of the new sensitization tests and treatment is made clear to the patient. The reviewer believes however that the author could safely have gone into greater detail in presenting in rather dogmatic fashion a layman's description of the immunologic principles involved.

In view of the large number of wheat sensitive individuals the recipes for wheat substitute breads, ten in number, will be most welcome.

In his discussion of the nature of Kapok pillows the author does not mention that these pillows are sometimes adulterated with small quantities of feathers. This, the reviewer has found in his own work to be a possible source of error of the greatest importance.

*Diathermy with Special Reference to Pneumonia. By Harry Laton Stewart M.D. Cloth. Illustrated. Pp. 228. Price \$3.00. Paul B. Hoeber Inc. New York. 1924.

†Hay Fever and Asthma. A Handbook for the Patient. By Ray M. Balyeat, A.M. M.D. Cloth. Illustrated. Pp. 193. Price \$2.00. F. A. Davis Company Publishers. 1924.

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS, MO., MAY, 1927

No 8

Editor-in-Chief WARREN T VAUGHAN, M D

Richmond, Va

ASSOCIATE EDITORS

DENNIS E JACKSON, M D	CINCINNATI
PAUL G WOOLLEY, M D	LOS ANGELES
I J R MACLEOD, M B	TORONTO
W C MACCARTY, M D	ROCHESTER, MINN
GERALD B WEBB, M D	COLORADO SPRINGS
VICTOR C MYERS, PH D	IOWA CITY
RUSSELL L HADEN, M D	ROSEDALE, KAS
JOHN A KOLMEY, M D	PHILADELPHIA
ROBERT A KILDUFFE, M D	ATLANTIC CITY, N J
A PARKER HITCHENS, M D	MANILA, P I
DEAN LEWIS, M D	- BALTIMORE
GEORGE R HERRMANN, M D	NEW ORLEANS
HAROLD D CAYLOP, M D	ROCHESTER, MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis Mo as Second Class Matter

EDITORIALS

Japanese Medical Education

IN THE fall of 1926 over 150 delegates visited Japan to attend the third Pan Pacific Science Congress in which the American medical world was so eminently represented by men like Dr Victor C Vaughan, and President Wilbur. During elaborate excursions the delegates were given an opportunity to make a somewhat hurried survey of Japanese scientific education and its accomplishments. What the writer saw at that time in the line of medical education is neither complete nor exact, but there were certain phases and tendencies in this work which were so strikingly different from most of ours that even a casual observer could not fail to take notice. These are recorded here at the suggestion of Dr D E Jackson.

The "full-time clinical professorship" is an indoor sport of American medicine. Before our medical schools have yet experimented with this system in toto, there are many physicians in America who are thoroughly convinced that it is an absolute failure. Japan has practiced this system for

years, full time men from departmental head to assistants in all colleges of medicine, nineteen in all. There are many collateral problems which are severely criticized by their own medical men. But there are several things which Japan has learned from the system. Among these are the following:

A college of medicine can be run in just the same way as a college of science or a college of arts. The men who teach in these colleges must be fundamentally investigators. The writer cannot name offhand a single example of a clinical professor in a medical college who does not hold a higher degree that was generally obtained by presenting a thesis based on some actual experimental scientific work. The most significant of all is the fact that those clinical men who hold the higher degrees are ranked as the highest of all clinical men in Japan. If we should ask a general practicing physician in Japan to name the first 100 most competent physicians and surgeons on the basis of clinical ability alone the writer will venture to predict that at least 90 per cent of those selected would be men who have research degrees. It is assuredly true and eminently demonstrated in Japan that so called "scientific medicine" does not prevent men from developing good clinical sense and judgment.

Not only are the clinical professors in Japan those who have in the past engaged in investigations of a fundamental and experimental nature but the contributions which continue to come from their own clinics are also of a thoroughly experimental character. One will be impressed with the titles of the articles contributed from these clinics. In a recent single number of a journal on experimental medicine having more than 500 pages, over 80 per cent of the articles were from a clinic on internal medicine in which were discussed such questions as internal secretions and gaseous exchanges of the blood and gaseous metabolism and blood flow to the brain under different conditions, the type of contributions one might well expect to come from physiologic or biologic laboratories. The recent monograph on intercellular oxidation and indophenol blue synthesis was written by the head of the department of internal medicine in a small medical college.

The conviction that research is the fundamental prerequisite for good clinical judgment and practice is so strong among the medical men in Japan that in most medical colleges there are at least 100 graduate students of medicine who are engaged in investigations in experimental medicine or in the preclinical sciences. These men are candidates for the higher degrees and are preparing for their careers by devoting their entire time to research. In one small medical college the writer counted at least fifteen doctors of medicine who were, as graduate students engaged in research in the biochemical department alone. The chemical nature of placenta toxins, the metabolism of cholesterol, the action of cholin and its derivatives are a few of their problems that the writer happened to remember.

The fact that no graduate student from a college of science was found in a medical school is interesting, but of no significance, being entirely due to a different educational system but the fact that so many young medical men are willing to spend three or four years in preparing for a higher degree by

doing pure research is exceedingly significant and should indicate the direction in which Japanese medicine is oriented

Our own medical colleges take just pride in their physical equipment which is the universal envy of the world in general and which is a very important factor in the development of medical education. But how to distribute rightly this physical equipment and the financial resources of a medical college between pure research and routine instruction is a much debated question. It was a matter of much difficulty for the writer to obtain exact data as to the actual proportion of this allotment in the Japanese colleges. He does remember, however, one instance in a physiology department in which 80 per cent of the floor space was devoted to the research laboratories and in which, nevertheless, one of the most satisfactory courses in physiology for medical students is reputed to be given. When he saw three string galvanometers in one department, he could not help but admire the wisdom of the executive, when the price of such an instrument and the limitation of financial resources were considered.

Japanese medicine has much to learn. When one of their prominent professors of surgery told a medical academy that "The equipment and the general methods of Japanese medical education are not far behind America" he was probably overenthusiastic about Japan. But what he failed to emphasize was the *type of contributions* made by their clinical men to medicine.

—Shuo Tashuo (D E J)

Some Clinical Tests for the Estimation of Circulatory-Respiratory Functional Efficiency

THE physiologic state, the so-called functional capacity, reserve or condition of the heart muscle, is generally accepted as the one great factor which practically alone determines the extent or limits of the physical activity of the individual at any time and, in a way, barring other disease processes, the span of one's useful existence. The problem of establishing this factor at all definitely is as difficult as it is important.

It is especially in the questionable or borderline cardiac cases, in which none of the reliable signs of heart disease are present, that one is usually desirous of obtaining some more satisfactory evidence of the integrity, the efficiency, resiliency, pliability or reserve of the circulatory-respiratory system. These cases sometimes present the symptoms of effort as dyspnea, palpitation, a labile pulse and blood pressure with a tendency to high levels on only slight exertion. Besides such cases of neurocirculatory asthenia which show no physical signs other than those of an irritable cardiovascular system, there are many individuals requiring further investigation, who have no complaints, but who have been found in periodical health surveys, employment applications or insurance examinations to have slightly suggestive signs, as systolic murmurs, changes in the character of heart sounds, deviations slightly beyond the established normal limits for blood pressure readings, or overactive hearts from the psychic effect of the examination. However, even in cases with reliable signs

of heart disease, but without evidences of congestive failure, it is often desirable from the prognostic standpoint to obtain accurate information of the status of the myocardium. The importance of the problem of devising a simple, reliable cardiovascular function test or tests is quite apparent.

Exercise tolerance tests with more or less crude standards of response in the rise of the pulse rate or blood pressure and the time necessary for return to the resting level have been in use for more than thirty years. These tests have their limitations, but are worthy of further consideration and standardization. The capacity for physical endurance is an objective criterion of the efficiency of the circulatory function and verifies and supplements the subjective opinion of the patient concerning his ability or limitation on exertion. The response to exercise often determines the management of the case, adding to the prognosis, as well as completing the cardiovascular study.

A system of *graduated tests* with the normal limits of response, such as have been outlined elsewhere¹ is of value. The tests increase in severity and the system should be carried only so far as the patient's condition will safely permit.

The observations consist in preliminary studies of the heart rhythm, the respiratory and heart rate counted for a full minute and recorded while the patient is at rest in bed or sitting at ease in a chair. The systolic and diastolic blood pressures are taken and recorded.

After each of the tests that follow, records should be kept of the extent of rise in the pulse rate and that in the blood pressures, delayed increase, no increase, or even a fall and the time necessary for the return to the resting levels determined. The degree of breathlessness and exhaustion produced and the occurrence of precordial or cardiac pain, rhythm changes, murmurs or shifting of the apex impulse should be noted.

THE GRADUATED SYSTEM OF CARDIAC FUNCTION TESTS

1. **Forced apnea (Russian)** Determine the length of time in seconds that the patient is able to hold his breath. Note any changes in the heart rate or rhythm during the test. The normal length of forced apnea is from thirty to sixty seconds and there is an accompanying moderate slowing of the heart rate. This is a gross index of the vital capacity and should be compared with spirometer readings. In effort syndrome cases and cardiac failure the forced apneic test period is rarely greater than ten seconds.

Cooper advocated the determination of the respiratory ratio, that is the ratio of the length of time that the breath could be held after deep inspiration and after complete expiration. Normally after deep inspiration the breath can be held forty to seventy seconds and after complete expiration twenty five to thirty five seconds. Variations from these ratios of 40/25 or 70/35 are suggestive of cardiorespiratory inefficiency.

Compression of the femoral arteries (Kritzenstein) This test has been used to increase the load on the heart and determine cardiac efficiency by the response. The compression may be accomplished by pressure with the thumbs, a tourniquet or a blood pressure cuff. The method has found very few supporters.

2 Pulling of interlocked hands above the head for two minutes (Dock) In this test there is normally a slight rise of ten to twenty points in the heart rate with a drop to the resting level within a minute

Heiz has suggested as tests other muscular maneuvers such as have passive movement against resistance, flexion and extension of the forearm, contraction of the muscles with or without extension of the lower limbs, and abduction and adduction of the thighs These tests, however, have not been generally used

3 Sitting up by the use of the abdominal muscles only and dropping back flat in bed five to ten times (Christian) This results in a rise of fifteen to thirty beats per minute and rise of as many millimeters of mercury in the blood pressure with a return to the resting level within two to four minutes Mendelsohn's test consisted in observing similarly the effects of a succession of rapid changes from the vertical to the horizontal position This test may be applied by having the patient bend forward ten times, attempting to touch the floor with his finger tips and coming back to the erect position with arms extended over the head

4 Walking more or less briskly for one or two hundred feet on level ground (Schott), or on slight inclines (Oertel), has long been in use as a functional test for patients who were up and about In order to provide for the accurate measurement of the amount of work done and its effect, Christ invented the steppage machine and attached a sphygmograph to the patient's wrist, recording the pulse rate graphically

5 The staircase rapid ascent (Selig) Normally, walking briskly up a flight of forty steps causes an increase in the heart rate of twenty to thirty beats per minute with a prompt drop to the resting rate within one minute, while the blood pressure does not rise more than 10 mm of mercury after this exertion In mild cases of the effort syndrome, the heart rate will increase to 120 to 130 per minute and in severe cases to 150 or 160 per minute and the fall to normal exceeds two minutes in duration The blood pressure likewise rises disproportionately Reactions in excess of these figures are evidences of myocardial insufficiency From the number of steps, the height of each, the incline and the individual's weight the amount of work can be calculated Running up and down the staircase has been suggested to bring out latent weakness of the heart muscle and can be employed in only a very select group

6 Hopping (Kahn) Twenty hops on each foot, raising the shoulder six inches each time, normally causes a rise of fifteen to twenty beats per minute in the heart rate and a rise of 5 to 10 mm of mercury in the blood pressure A drop to resting levels normally occurs within two minutes There may be considerable variation if the patient's weight and his amount of cooperation are not taken into account

7 Squatting (Stroud) With heels together and toes far apart, bending the knees as far as possible and coming back to the erect position fifteen times in a half minute's time will cause in a normal individual a rise of twenty-five to thirty heart beats per minute and a blood pressure increase of

10 to 15 mm of mercury, with a return to within five points of the resting level after two minutes. Most normal subjects are moderately breathless after this test.

In selected cases the number of squats can be safely increased to twenty or thirty performed within a minute.

8 Stepping up (Sehneider) on a chair twenty times that is, placing one foot squarely upon a chair seat eighteen inches from the floor and raising the body to the full erect position increases the heart rate in normal individuals twenty five to forty beats per minute with a rise in blood pressure of 10 to 15 mm of mercury and a return to normal in two minutes. Practically all normal individuals show breathlessness after this test.

9 Lifting dumb bells (Barringer) two fifteen pounders, from the floor to the full length of the arms above the head twenty times in forty seconds causes in normal individuals a rise of forty to fifty heart beats per minute and an increase of 20 to 30 mm of mercury in the blood pressure which drops to within ten points of the resting levels within two minutes. Considerable breathlessness is provoked in a fourth of the normal individuals by this amount of work.

Lifting two twenty pound dumb bells, through six feet thirty times in sixty seconds, raises the heart rate fifty to sixty beats per minute and the blood pressure 20 mm of mercury. Lifting the twenty pound dumb bells sixty times in one hundred and twenty seconds increases the normal individual's heart rate sixty to eighty beats per minute and the blood pressure rises as much as 30 mm of mercury.

Very often the effort syndrome case is unable to lift the twenty pound weights more than ten times and even with this amount of work his heart rate increases sixty to eighty beats and his blood pressure rises as much as 50 mm and the drops within two minutes do not reach the resting levels by twenty to twenty five points. Myocardial insufficiencies often show a delayed rise in blood pressure after these maneuvers.

10 Standardized work recording machines (Zuntz Graupner Wolffe) Ergostats or ergometers on stationary bicycles or weight and pulley apparatus for automatically measuring the amount of work done in producing certain effects have been devised but all have had definite limitations of practicability.

Wolffe⁴ has recently advocated the use of a cardiovascular dynamometer built along the lines of a brake binder which is rotated by the patient against the friction resistance by means of handles. The formula is derived and a work chart is drawn. The force in pounds is kept constant at ten to twelve for a given study and the number of revolutions is 50 to 100 with 3000 to 6000 foot pounds of work. The average rise in heart rate for 6000 foot pounds of work was thirty five beats with a return to the resting level in four minutes.

THE CARDIO RESPIRATORY TEST FOR CIRCULATORY EFFICIENCY

Abnormal variations in intrathoracic pressure have been found by Frost² to produce blood pressure reactions. These he considers to be definitely dependent upon the integrity of the cardiovascular system indicating the severity of

the strain and the efficiency with which it is resisted. An adaptable test has been devised in which a predetermined strain could be measured and the reaction recorded. A test such as this can be applied and months or years later reapplied under approximately the same conditions and at approximately the same degree of severity. This allows direct comparisons of reaction from time to time to determine the degree of progress of degenerative changes. The test is easily and safely applied to the aged and frail as well as to the young and sturdy, and permits observation of the response in the blood pressure, cardiac rate and rhythm during the application of the strain.

The apparatus* required is simple, compact, hygienic and easily portable in a small bag. The vital capacity readings with this type of windwheel spirometer are not absolute but relatively accurate and comparable. A blood pressure apparatus is also required. The gauge and spirometer are connected by means of a hard rubber Y tube and rubber tubing with a hard rubber stop cock inserted between the one branch of the Y tube and the spirometer while to the trunk of the Y tube is attached a piece of rubber tubing carrying the demountable sterilizable glass mouthpieces.

Of the nine steps or determinations, the first and ninth consist in control observations at the beginning and at the end of the test. In the second and fourth steps, increased intrathoracic pressure is produced by holding a full inspiration and by exhaling against the gauge. In the third and fifth steps decreased intrathoracic pressure is produced by holding a forced expiration and by inhaling against the gauge. In steps six, seven and eight increased intrathoracic pressure is produced and maintained through the greater part of the three exhalations and the approximate vital capacities are recorded by the spirometer.

TECHNIC, REACTIONS, INTERPRETATION

Step 1 Preliminary physical examination with especial reference to the blood vessels and the heart. The heart rhythm and rate are noted and the systolic and diastolic blood pressures are determined and recorded. It is advised to tuck the bowl of the stethoscope under the edge of the blood pressure cuff in order that the examiner's hands be free for manipulation of the apparatus. In practice only the systolic blood pressure in the significant part of each reaction is all that can be hoped for by one examiner.

Step 2 (Full inspiration held) The systolic and diastolic pressure are taken and recorded and the subject is then instructed to inhale as deeply as possible and hold the inspired air in for ten seconds.

The air of a full inspiration should be retained by closing the glottis and allowing the chest and diaphragm to relax against the inflated lungs, the pressure of the relaxed walls producing the increased intrathoracic tension.

At the end of the inhalation the systolic, and if there is a second observer and apparatus, also the diastolic blood pressure are taken at least once but preferably twice, early and late, in the ten second apneic period.

*The outfit is furnished by the Taylor Instrument Company of Rochester, New York and consists of a Tyco's vacuum-pressure gauge and a Simplex windwheel spirometer.

In normal individuals synchronous with this increase in intrathoracic pressure there is for two or three seconds, a quick initial rise averaging 5 mm systolic pressure, then a rapid decline of 20 mm or more, so that the systolic appeared to merge with the diastolic pressure the level of which usually rises 10 to 15 mm. In the later stages of Step 2, as the systolic rises rapidly toward its resting level the diastolic pressure drops back toward its level. Both effects carry the levels a few points beyond the baselines as the air is released from the lungs.

The significant part of the reaction is considered to be the maximum decline of the systolic pressure. The systolic blood pressure fall should be at least 10 mm of mercury and may drop in young adults until it merges with the diastolic. A failure to fall has been observed to occur in individuals with more or less rigid arterial systems and powerful hearts.

Step 3 (Full expiration held) After a rest of ten seconds the systolic and diastolic pressures are again determined. The subject is instructed to exhale as far as possible and to refrain from inhaling for ten seconds.

The air should be excluded by closing the glottis and relaxing the chest and diaphragm against the deflated lung. The suction thus produced results in a condition of decreased intrathoracic tension.

At the end of the expiration the systolic and if there is a second observer and apparatus also the diastolic blood pressure are taken at least once but preferably twice, early and late in the second period just as unhalation is beginning.

In normal individuals synchronous with the decrease in intrathoracic pressure there is for about five seconds an initial decline in systolic pressure averaging 5 mm of mercury, and then a gradual rise to 5 to 10 mm. above the resting level. In the later stages of Step 3 as the systolic rises the diastolic pressure falls 4 to 10 mm which seems most characteristic of this step, and then gradually returns to normal. As inspiration is begun there is usually a quick increase in pressure up to 20 mm above the original level, then a gradual decline to the baseline.

The significant part of the reaction is considered to be the maximum rise in the systolic pressure. The systolic blood pressure rise of more than 20 mm of mercury above the resting level is taken to indicate an irritable unstable overacting, hyperactive cardiovascular system responding to strain with an excessive expenditure of energy.

Step 4 (40 mm positive pressure held) After a rest of ten seconds the systolic and diastolic pressures are again determined. The subject is instructed to blow against the Tycos gauge, with the spirometer cut off by means of the stopcock, and to maintain a positive pressure of 40 mm of mercury for about ten seconds.

The positive pressure is to be maintained by the chest and diaphragm rather than by the buccal muscles. A condition of increased intrathoracic pressure is produced similar to that in Step 2. The observations necessary,

the reaction, significant point and interpretation are similar to those given for Step 2

Step 5 (25 mm negative pressure held) After a rest of ten seconds, the systolic and diastolic pressures are again determined. The subject is instructed to draw in against the gauge, the spirometer remaining cut off, maintaining a negative pressure of 25 mm for about ten seconds.

The negative pressure is to be maintained by the chest and diaphragm rather than by the buccal muscles. A condition of decreased intrathoracic pressure is produced similar to that in Step 3. The observations necessary, reaction, significant point and interpretation are similar to those given for Step 3.

Steps 6, 7 and 8 (Expiration to full capacity through spirometer at 20 mm positive pressure) After ten second intervals the control blood pressures, systolic and diastolic are taken before each of these three similar maneuvers.

The stopcock in the tube to the spirometer is opened and the subject is instructed to inspire as deeply as possible and then to blow as long as possible through the spirometer. He must maintain a constant positive pressure of 20 mm of mercury throughout by watching the gauge and keeping the indicator at the 20 mm mark.

The blood pressure fluctuations are followed as in Step 2. The approximate vital capacity as indicated by the spirometer is recorded. The test is repeated twice in the same manner.

A condition of increased intrathoracic pressure is produced and maintained through the greater part of the expiration though of necessity gradually declining towards the end.

In normal individuals in the early stages of these tests reactions similar to those obtained in Steps 2 and 4, a quick initial rise followed by a rapid fall are noted. Later toward the end of expiration the systolic pressure began to rise, reaching 20 to 40 mm above the resting level with frequently an added 5 to 10 mm. at the end just as inspiration began and then a rapid and later a gradual decline. The diastolic pressure followed as in Steps 2 and 4 rising at first and falling 5 to 10 mm below the baseline just after the systolic peak was reached.

The significant part of the reaction is considered to be the maximum rise in systolic pressure. The systolic blood pressure should rise at least 20 mm and at the most 50 mm above the resting level. Failure to rise at least 20 mm is considered an indication of a weakened myocardium or valvular obstruction, while a rise of more than 50 mm is taken as an indication of an irritable, overactive cardiovascular system or an abnormally powerful heart.

Step 9 After about thirty seconds rest, the final observations are made. The systolic and diastolic blood pressures, the heart rate and rhythm, and the respiratory rate are noted. The systolic blood pressure is usually 5 to 10 mm above the original level, the heart rate is as a rule increased 5 to 10 beats per minute, but frequently baseline or slightly lower final figures are obtained.

SUMMARY OF THE REACTION

Step 1 Resting Control

2 and 4 Increased Intrathoracic Pressure

Systolic BP fall at least 10 mm

Failure to fall suggests arteriosclerosis Diastolic BP rise 10 mm

2 and 5 Decreased Intrathoracic Pressure

Systolic BP drops 5 mm at start then rises 5 to 10 mm above the resting level. A rise of more than 20 mm is taken to indicate an irritable overactive cardiovascular system. Diastolic BP fall 4-10 mm

6, 7, and 8 Increased Intrathoracic Pressure

Systolic BP as in 2 and 4 shows a quick initial rise followed by a rapid fall

Later towards the end of expiration the systolic BP rises 20 to 40 above the baseline. A minimum of 20 mm and a maximum of 50 mm increase. Failure to rise 20 mm is taken to indicate a weakened myocardium or valvular obstruction while a rise of more than 50 mm indicates an irritable overactive heart. Diastolic BP shows a slight initial rise then a fall 5-10 mm then a rise. The base line fluctuation is only 5-10 mm in normal individuals

Step 9 Final Control

COMMENTS

The cardiorespiratory test, as described by Frost, is certainly a most promising clinical method of estimating the functional state of the circulatory system. The many advantages of the test have been enumerated in the opening paragraphs.

A mechanical method of recording the blood pressure fluctuations continuously throughout the entire period of strain would simplify matters considerably and at the same time add an important graphic check. Considerable practice is required in the use of the manometer bulb and the release valve before one can follow accurately the rapid fluctuations in the blood pressure during the test. Frost says that after a little experience, however, the examiner knows intuitively the direction in which the pressure will fluctuate and will follow it more alertly. The taking of systolic pressures in the most characteristic and striking phase of the reaction is, however, all that one examiner can hope to accomplish accurately. The pressure must be released from the cuff after each test.

It is sometimes difficult to get a patient to understand the maneuver that he is to perform in Steps 2 and 3, but since the changes effected are accomplished by simpler subsequent procedures, there is adequate corroboration and

duplication The method may bear even further simplification Further studies with accompanying graphic methods, especially in cases with the reliable signs of heart disease, are necessary and are no doubt in progress in various laboratories The results of these investigations will help to establish the final status of this clinical cardio-respiratory test

REFERENCES

- ¹Herrmann, George R Methods in Medicine, 1924, p 189, C V Mosby Co, Exercise Tolerance Tests
- ²Frost, H M Life Insurance Medicine, 1926, 1, 136, (New England Life Insurance Co), The Cardiorespiratory Test, Boston Med and Surg Jour, 1924, cxc1, 853
- ³Kahn, M H The Functional Diagnosis of the Heart, Med Clin N A, September, 1919, iii, 433
- ⁴Wolffe, Jos B Cardiac Capacity Determined by Standardized Effort, Arch Int Med. December 1926, lxxviii, 761

—G R H

The Journal

LIBRARY RECEIVED
JUN 11 1927

of
U.S. Department of Agriculture

Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., Editor in Chief
Medical Arts Building
Richmond Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS D. JACKSON, M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, M.D.
University of Toronto Toronto

Clinical Pathology

W. C. MACCARTY, M.D.
Mayo Clinic, Rochester Minn.

Biochemistry

VICTOR C. MYERS, Ph.D.
State University of Iowa, Iowa City

Immunology

JOHN A. KOLMER, M.D.
University of Pennsylvania,
Philadelphia

Bacteriology

A. PARKER HITCHENS, M.D.
Bureau of Science, Manila, P. I.

Internal Medicine

GEORGE R. HERRMANN, M.D.
Tulane University New Orleans

Pathology

PAUL G. WOOLLEY, M.D.
Los Angeles

Tuberculosis

GERALD B. WEBB, M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR, M.D.
Mayo Clinic Rochester Minn.

Experimental Medicine

RUSSELL L. HADEN, M.D.
University of Kansas Rosedale

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY 323 25 PINE BLVD. ST. LOUIS.

Entered at the Post Office at St. Louis Mo. as Second Class Matter

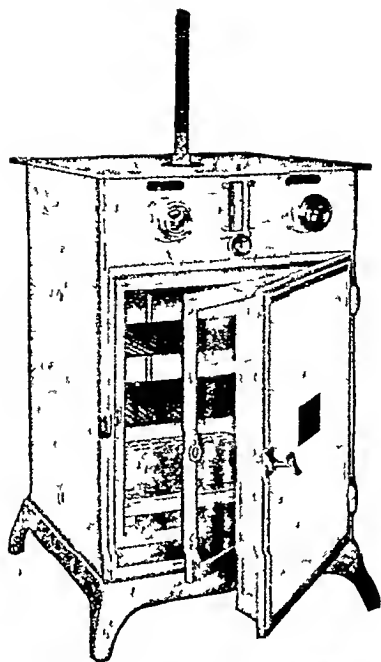
LABORATORY EQUIPMENT

Purchasing agents for private or college laboratories are requested to write us for prices when needing equipment. Estimates Cheerfully Furnished. Prompt Service.

See our advertisements, pages 26 and 27

EDWARD P. DOLBEY & COMPANY

PHILADELPHIA, PA.



Freas Incubator No. 26

Your Incubator Must be Accurate

Just as with your laboratory worker your incubator must be capable of performing the assigned tasks accurately

Accuracy in an incubator is its ability to maintain the temperature constant continuously

This is why the Freas Incubators are used in the leading laboratories

The Thermo Electric Instrument Co.
1215 South Grove Street, Irvington, N. J.

FREAS

ELECTRIC INCUBATORS

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO, JUNE 1927

No 9

CLINICAL AND EXPERIMENTAL

EARLY INFANTILE PROGRESSIVE MUSCULAR ATROPHY (WERDNIG HOFFMANN) A CLINICAL AND PATHOLOGIC STUDY OF TWO CASES*

BY CHARLES E NIXON M D, AND JEAN OLIVER, M D SAN FRANCISCO, CALIF

THE Werdnig Hoffmann type of progressive muscular atrophy is a comparatively rare disease, a search of the literature shows a record of about twenty typical cases, and of these only ten were studied from both the clinical and pathologic side. There is therefore still considerable uncertainty, not only as to the classification of the different types of muscular atrophy in infants, but even as to whether or not the syndrome described by Werdnig¹ and by Hoffmann² is a clinical entity. In a recent paper Huenekens and Bell³ come to the conclusion from a review of the literature and a study of the case reported by them that amyotonia congenita (Oppenheim) and infantile spinal progressive muscular atrophy (Werdnig Hoffmann) are extreme types of the same disease and that they are probably both related to the groups of myopathies represented by Erb's juvenile form of muscular dystrophy and the hereditary form of Leyden and Mobius.

Another obscure point which the recorded cases have not made clear is the relation of degeneration in the cardiac muscle to the extensive lesions that occur in the skeletal system. Globus⁴ has recently reported an instance of cardiac involvement in a case of progressive muscular dystrophy and has reviewed the literature of this phase of the subject. He found a fragmentation of the muscle cells with multiplication of their nuclei and an infiltration of the interstitial tissue with fibroblasts. In the descriptions of the older writers the heart was either not examined or no detailed descriptions are given of the microscopic findings.

In the first case reported in this paper the spinal cord and muscular

*From the Department of Pathology University of California Medical School and the Department of Pathology Stanford University Medical School.

Received for publication Nov 29 1926

involvement are of the characteristic type without any lesions in the heart muscle. The second case shows a remarkable type of acute cell change in the anterior horn cells and definite and equally acute involvement of the heart muscle.

CASE 1—V O, aged three months. The family history is negative except that one well developed child was stillborn. The birth was spontaneous by breech presentation but not prolonged. A marked deformity of the chest was present from birth and respiration was diaphragmatic and abdominal. The arms were paralyzed, the right more seriously than the left. There was a double wrist drop with contractures. Otherwise the limbs were flaccid. The right foot was in the position of a moderate equinovarus and the left in a moderate calcaneovalgus. At the time of examination in the Children's Hospital, when the child was two and one half months old, the deep reflexes were entirely absent. There was no glandular enlargement and the von Pirquet and Wassermann tests were negative. The blood count was normal. The x-ray plate of the skull was negative.

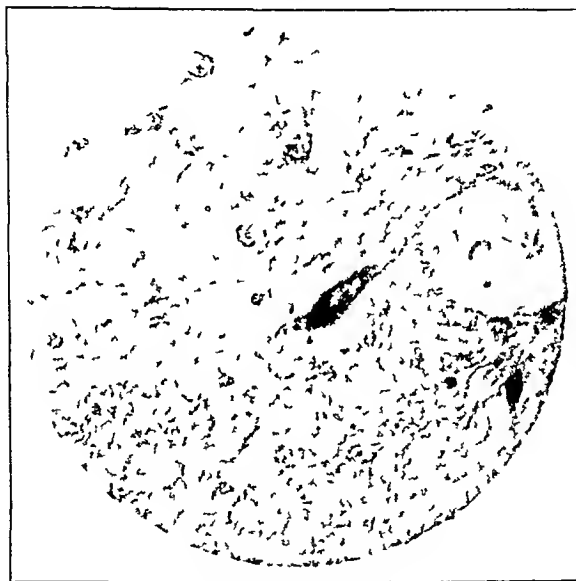


Fig 1 (Case 1) Anterior horn cell

The essential necropsy findings are as follows. The body is that of a poorly developed and nourished white male infant of three months. The pectoral muscles are firm and apparently fibrous. The upper half of the chest is small and depressed. The lower half shows marked flarings of the costal margin. The costochondral junctions are fairly prominent. The muscles of the extremities are all atrophic. The arms are partially flexed and complete extension of the elbows is not possible. The thymus is fairly large. The skull was not opened.

Microscopic Examination—The anterior horn cells of the cord are strikingly smaller than normal, there is not, however, a definite diminution in number. The cell changes are most marked in the cervical and thoracic regions but are also present in the lower cord. In the thoracic cord the lateral cells belonging to the visceromotor groups are much more normal in appearance than the other anterior horn cells.

The type of cell change is uniform throughout the cord and the extent of the cell alteration varies only to a moderate degree. The characteristic anterior horn cell is spindle shaped, the nucleus is relatively large and pale, the chromatin substance is more or less clumped, and as a rule at one end of the cell (Fig 1). The anterior horn cells of the lum

bar cord show less change the cells are larger and somewhat resemble a normal cell but the nucleus is comparatively large and pale and the cytoplasm and arrangement of the Nissl bodies definitely varies from the normal. The chromatin substance is either in a few large clumps or is situated peripherally leaving the cytoplasm largely clear.



Fig. 2 (Case 1) Anterior root Weigert's method stain



Fig. 3 (Case 1) Posterior roots Weigert stain

The cells of Clarke's column show moderate chromatolytic changes. The nucleus is eccentrically placed, often appearing to be partly extruded from the cells. The chromatin material tends to be clumped in one end of the cell. In a few cells the chromatolysis is extreme so that the cell consists of a swollen nucleus partly extruding from a small amount of granular cytoplasm.

There are no special changes in the glia tissue, satellites and the so called neurophagia are occasionally noted, somewhat more in Clarke's column than in the anterior horn. No gutter cells are seen.

Weigert sections of the cord show a fairly marked degeneration of the anterior roots, the posterior roots are normal (Figs 2 and 3).

The somatic muscles are extremely involved (Fig 4). There is great variation in the size of the fibers. The fasciculae may be made up wholly of small fibers or of both large and small fibers. In some of the muscle sections the cross striations are indistinct. Vacuolization is occasionally seen. Pigmentation is present in some of the bundles and varies from yellowish to yellowish brown in color. The heart muscle appears normal.

CASE 2—We are indebted to Drs F. Sylvester and Langley Porter for the details of the clinical history of the case.

A Japanese baby, five months old, was admitted to Lane Hospital on November 1st. It had been well until two weeks previously when it was noticed that its legs were swollen.

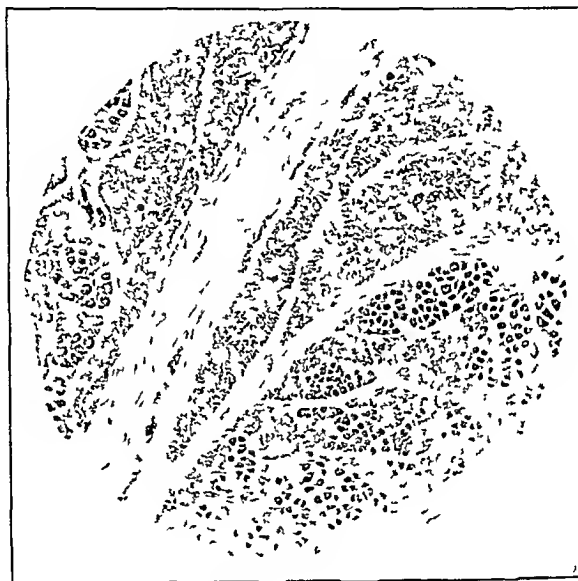


Fig 4 (Case 1) Skeletal muscle

Shortly after this time Dr. Sylvester was called and noted a flaccid paralysis of practically all the muscles below the head. Even the muscles of deglutition were affected, making tube feeding necessary. A week later the child developed difficulty in breathing. On admission to the hospital the baby was limp and pale and frothing at the mouth. It rallied somewhat after a mustard bath, but the dyspnea increased and the child died the morning following its entrance to the hospital. The urine was normal and there was no disturbance in the digestive tract. A diagnosis of progressive muscular atrophy was made.

Pathologic Examination—The necropsy was performed three hours after death. The body was that of a rather emaciated normally formed male child of normal size for its age. There was a marked atrophy of the subcutaneous fat. There was a moderate elastic edema of the palms of the hands and the soles of the feet and a marked edema of the scrotum. No special atrophy of any group of muscles was evident, but sections of the muscles in various places showed a definite atrophy of them and they were pale in color.

The peritoneal cavity was empty. All the abdominal organs were in normal position. Outside of a marked congestion they showed no abnormalities.

The level of the diaphragm was the fourth rib on both sides. The thymus was not

enlarged and was normal on cut section. There was about 25 c.c. of clear fluid in the pleural cavities.

Both lungs showed subpleural hemorrhages scattered over the surface of all lobes. The posterior portions of all lobes were airless and on section showed areas of collapse and of consolidation. The peribronchial lymph nodes were swollen and edematous.

The head was of normal size. The parietal eminences were somewhat prominent and the forehead flat. The anterior fontanelle was open, measuring 1 cm. transversely and 2 cm. longitudinally. The skull was normal. The external surface of the dura was normal.

There was a marked edema of the pia over the convexity of the cerebral hemispheres, the convolutions appeared normal. The general external configuration of the brain was normal. In the pia at the base, beginning at a point 1.5 cm. below the lower edge of the pons was a diffuse infiltration of the pia with recently shed, poorly clotted blood. The hemorrhages extended laterally about 0.5 cm. over the adjoining portions of the cerebellum.



Fig 5 (Ca e 2) Skeletal muscle

Transverse cut sections through the brain and brain stem showed no gross lesions. The hypophysis was normal. The large venous sinuses at the base of the skull were normal.

The venous plexus around the cord was greatly congested. There was a marked edema of the loose connective tissue around the cord, especially in the lower portion of the spinal canal and a considerable collection of spinal fluid within the dura. The dura and pia on both sides of the cord were normal. The cervical and lumbar enlargements were well developed. Cross sections of the cord in representative regions showed no gross lesions.

Smears of the consolidated portions of the lungs showed many polymorphonuclear leucocytes and a great number of gram positive diplococci.

Histologic examination of the kidney, liver, pancreas, thymus, peribronchial lymph glands, stomach, large and small intestine and spleen showed no abnormalities except congestion. Sections of the lung showed the alveolar spaces filled with exudate and leucocytes.

To summarize, the examination, other than that of the nervous system which will be given in detail later, showed a general atrophy of the skeletal muscles including the heart muscle, and a bronchopneumonia. Death was evidently due to the latter and to the circula-

tory failure, evidenced by the marked dilatation of the heart, the marked congestion of the venous system and the edema

Examination of the Nervous and Muscular Systems—Sections of striated muscle were examined from representative groups, including the muscles of the leg, chest wall and abdomen and diaphragm. In all the sections the lesions were of the same degree and character, so that a single description will cover the pathologic lesions in all

There is an almost complete disruption of all the muscle cells in every section and the various stages in the process of disintegration can be clearly followed. The least degree of damage consists in a swelling of the muscle cell to perhaps twice its normal size. The cross striation can still be made out. As the process becomes more severe these markings disappear and the cells take on a diffusely granular appearance. Vacuoles appear which with Sudan III are found to be filled with fat. This solution of the protoplasm of the cell allows structures to become visible which are not seen under normal conditions. This is particularly true of the sarcostyles of the muscle cell which are more resistant and therefore persist long after the remainder of the muscle cell is transformed into a granular mass. But

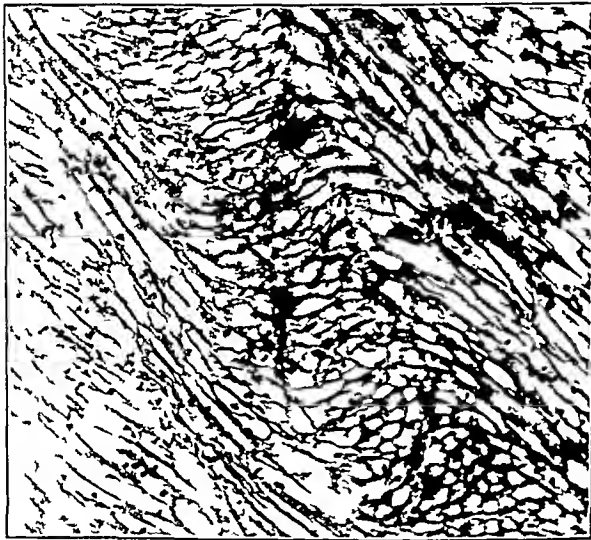


Fig 6 (Case 2) Cardiac muscle

these structures also ultimately degenerate and the cell becomes a bag like structure consisting of granular material and fat droplets contained in the intact sarcolemma (Fig 5)

The changes noted in the cardiac muscle were equally severe, but of a somewhat different character. In them the change was principally a vacuolar one, all stages of the process from the appearance of a few small fat containing droplets to complete transformation of the cell body into one large cavity, surrounded by a thin zone of granular protoplasm in which no fibrillae can be seen (Fig 6). Practically every cell in sections from all parts of the heart were involved.

Sections from different parts of the nervous system were stained with the following methods. Van Gieson, hematoxylin and eosin, methylene blue, Giemsa, Weigert Pal and Marchi.

The Peripheral Nerves—No lesions could be seen with either Van Gieson's or hematoxylin and eosin stain in the peripheral nerves either from the brachial and lumbar plexus or in the finer ramifications contained in the sections of the muscle. With Marchi's method a slight degeneration was found in all those examined. Scattered between the nerve fibrils were a few small droplets from the degenerating myelin sheaths.

The Spinal Cord and Brain Stem—The most extensive lesions found in the nervous

system were in the spinal cord and here the pathologic change was most marked in the motor cells. These cells present a most remarkable appearance. Throughout the entire cord all the motor cell groups and the cells of Clarke's column showed the same changes. These



Fig. 2 (Case 2) Anterior horn II with proximal myopathy (same magnification as in Fig. 1)

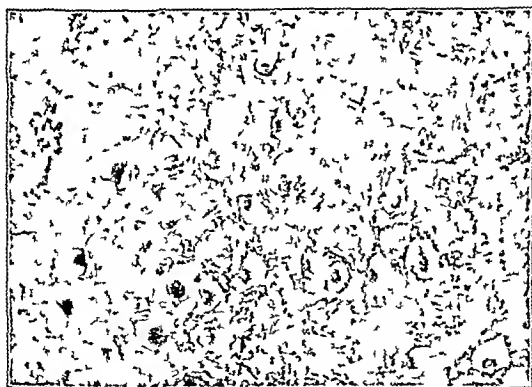


Fig. 3 (Case 1) Group of anterior horn cells in lumbar region

consisted of a marked swelling of the cell body, a solution of the tigroid material and eccentricity of the nucleus. The clear protoplasm consisted of a delicate network which became progressively more attenuated as the distance from the nucleus increased (Fig. 7). A group of these pale swollen cells from the lumbar region is shown in Fig. 8.

The same cell changes were also present in the nuclei of the medulla and brain stem.

The nuclei of all the cranial nerves, the olives and dentate nucleus of the cerebellum and the nuclei of the pons all showed the changes to an almost equal degree

In the midbrain the same degeneration of the ganglion cells was also noted, though to an appreciably lesser degree, while in the cerebral cortex the pyramidal cells were entirely normal

In sections stained by both the Marchi and Weigert-Pal methods for degeneration of the medullated fibers no definite lesions were found in any part of the central nervous system. Nor was there any evidence of proliferation of glia or of any inflammatory process such as perivascular round cell infiltration or hemorrhages

The pathologic changes in the nervous system can therefore be summarized as consisting of a widespread degeneration of the ganglion cells of the lower motor nerves with no evidence of tract degeneration or of any inflammatory process. The peripheral nerves are normal and there is an extensive degeneration of the skeletal muscle and the cardiac muscle. The degeneration in both the nervous and muscular systems is of the type commonly associated with acute processes

Two points are worthy of comment in this case. In the first place the rapidity of the clinical course of the disease is in marked contrast to the protracted course, extending over several months or even years which has been observed in the majority of cases studied. The only similarly rapid case which we have found in the literature is one described by Balton, where the duration was three weeks. The anatomic changes also differ from those previously studied. In them the lesion is described as consisting of an atrophy, shrinkage and disappearance of the motor ganglion cells of the spinal cord, whereas, these cells in the present case show degenerative changes but of the so-called "acute" type, swelling of the cell, chromatolysis, irregularity and eccentricity of the nucleus. Our report therefore adds to the literature an acute case of progressive spinal muscular atrophy of children.

The second point of interest is the extensive involvement of the myocardial muscle.

SUMMARY

This paper is a clinical and pathologic report of two cases of early infantile progressive muscular atrophy of the Werdnig-Hoffmann type.

One case presents the usual pathologic findings of this disease consisting of marked atrophy of the anterior horn cells of the spinal cord and moderate chromatolysis of Clarke's column cells without evidence of an inflammatory process, the somatic muscles show extensive atrophic changes.

The second case is remarkable for the acute type of change in the anterior horn cells and the striking involvement of the heart muscle.

REFERENCES

- ¹Werdnig Arch f Psychiat, 1894, xxvi, 706 Ibid, 1891, xxii, 437
- ²Hoffmann Ztschr f Nervenhe, 1897, x, 292
- ³Huenekens and Bell Am Jour Dis Child, 1920, xv, 496
- ⁴Globus Arch Neurol. and Psychiat, 1923, ix, 59

THE ORGANIC PHOSPHORUS OF THE CEREBROSPINAL FLUID*

By GUY E. YOUNGBURG, PH D, BUFFALO N Y

INTRODUCTION

WHILE the amount of inorganic phosphorus of cerebrospinal fluids has been rather definitely determined during the last several years and concordant and consistent results obtained by a number of different workers,^{1 2 3 4} the question of the occurrence and quantity of organically bound phosphorus has been given only slight attention.² The reasons for this are obvious, viz first, the small amount of organic phosphorus, and secondly, unsuitable analytic procedures.

Mestrezat,⁵ Donath,⁶ Apelt and Schumm,⁷ Williamson⁸ and others, have found organic phosphorus. Hauerwitz concludes from his work that all of the phosphorus of the cerebrospinal fluid is in the inorganic form.

In consideration first of the possible clinical significance that might be attached to an increase or decrease of organic phosphorus through migration or degeneration of nerve tissue (phosphatides) of the central nervous system, or of products therefrom, or even phosphoproteins, in such disorders as tuberculous meningitis, neurosyphilis, lethargic encephalitis etc, and secondly, in consideration of the now greatly improved micromethods for phosphorus determination notably that of Benedict and Thies,⁹ I have undertaken to obtain figures for the organic phosphorus of a large number of cerebrospinal fluids from patients with various disorders.

Although nerve tissue changes would be expected to be slow yet it is not out of the question that such changes might be great enough to be reflected in the phosphorus content of the fluid.

Analyses for inorganic phosphorus were also made until it became evident that the content of inorganic phosphorus is entirely independent of the content of organic phosphorus, or vice versa.

ANALYTIC METHODS

Organic phosphorus was determined by precipitating inorganic phosphates with magnesia mixture, digesting the evaporated filtrate with sulphuric and nitric acids in the presence of a little copper sulphate and estimating the phosphorus by a modification of the Benedict and Thies⁹ colorimetric method.

Special reagents used

Magnesia mixture—The widely used solution containing 55 gm magnesium chloride, 70 gm ammonium chloride and 88 cc of conc ammonia water per liter was employed.

*From the Biochemical Laboratories of the Buffalo City Hospital and the University of Buffalo Medical School.

Received for publication December 3 1936

Ammonium molybdate—3.1 per cent solution in water The salt was purified by the Bell-Doisy¹⁰ method

Hydroquinone bisulphite—0.5 gm hydroquinone and 15 gm sodium bisulphite per 100 cc of water solution

Standard phosphate—Solutions of KH_2PO_4 , 5 cc equivalent to 0.0025 mg and 0.005 mg P

Procedure—Three cc of cerebrospinal fluid was transferred to a small test tube, 0.25 cc magnesia mixture was added, and after mixing, was allowed to stand from six to eighteen hours The solution was then filtered into a 15 cc Pyrex test tube graduated at 10 cc, the filter paper washed with several cc of water and the contents of the tube evaporated to dryness over a hot plate 0.6 cc of sulphuric acid, 1 drop 10 per cent copper sulphate and 2 silica pebbles were then added and the tube was heated with a micro burner When white fumes appeared a drop or two of dilute nitric acid (1 to 10) was added If the contents did not decolorize upon heating again, more nitric acid was added Heating was continued until all of the nitric acid was driven off, without at the same time losing any of the sulphuric fumes After cooling, 5 cc of water, 2 cc of ammonium molybdate and 1 cc of hydroquinone-bisulphite were added, mixing after each addition, and the solution was made up to the mark and mixed

Standards containing 0.0025 and 0.005 mg P were heated to boiling with 0.6 cc sulphuric acid and 1 drop of copper sulphate as above and completed as for the unknowns Color comparisons were then made in a colorimeter

Blank determinations must give but a trace of blue

Notes on above method Magnesia mixture is widely used as a complete phosphate precipitant and it has been found in this laboratory¹¹ to be the most efficient for such a purpose as this

While it would be desirable to develop more color, it is often difficult to obtain more than 3 cc of cerebrospinal fluid when quantities for routine tests must first be taken The amount of color obtained by the Benedict and Theis procedure is, however, astonishingly great for a very small amount of phosphorus and the determinations reported were made with sufficient accuracy although they are only to be considered as approximate values

The Benedict and Theis method was used for the determination of inorganic phosphorus

EXPERIMENTAL

Cerebrospinal fluids were obtained as they came to the general laboratories of the hospital for the usual determinations They were from both ward and out-patient departments and represent various disorders None of the fluids used contained any visible traces of blood, although three showed red blood cells

Globulin, cell count colloidal gold and Wassermann tests were made as routine work and the phosphorus determinations as special work The results on 200 fluids were obtained, but for the sake of brevity only the results of 20 typical ones are presented in Table I.

TABLE I
SHOWING ANALYSES OF 20 TYPICAL CEREBROSPINAL FLUIDS FROM HOSPITAL AND OUT PATIENT CASES

NO	GLOBULIN	CELLS	WASSERMANN	ORGANIC P	INOORGANIC P	FINDINGS
		No per cmm		Mg per 100 cc	Mg per 100 cc	
1	4+	210	+	0.59	4.19	
2	4+	116	4+	0.23	1.07	
3	3+	72	4+	0.13	1.97	
4	3+	14	4+	0.15	1.58	
5	3+	Too numerous	Anticomp	0.41	1.35	A purulent fluid (pneumococcus)
6	2+	13	4+	0.11	1.35	
7	2+	33	4+	0.29	1.27	
8	1+	5	4+	0.12	1.51	Previous lactic gold curve
9	1+	163	-	0.17	0.10	
10	1+	84	4+	0.06	1.31	
11	1+	7	-	0.19	1.63	
12	1+	4	4+	0.20	0.97	
13	trace	4	-	0.14	1.33	
14	-	12	-	0.13	1.26	
15	-	1	4+	0.10	1.6	
16	-	8	-	0.13	1.26	
17	-	1	-	0.14	1.28	
18	-	1	4+	0.16	1.30	
19	-	3	4+	0.18	1.38	
20	-	2	-	0.21	1.39	Acute encephalitis Patient 13 years old

Surplus fluids were pooled and evaporated to dryness below 75° C. Five gm of solids were extracted with 100 cc of warm alcohol-ether (redist alcohol 3, redist ether 1), filtered, digested with acid and phosphorus determined as indicated under analytic methods. The object was to determine lipid phosphorus. Since this was found to be practically nil the phosphorus was probably present as a constituent of protein and the fluids have thus been arranged in the order of decreasing amounts of globulin.

Table II gives determinations of organic phosphorus on five postmortem fluids. This work was done to find if there is a rapid, slow, or no influx of organic phosphorus compounds after death, or if these compounds split off phosphoric acid readily.

TABLE II

SHOWING AMOUNT OF ORGANIC PHOSPHORUS IN POSTMORTEM CEREBROSPINAL FLUIDS

NO	HOURS AFTER DEATH	ORGANIC P MG PER 100 CC	PATHOLOGY
1	1	0.10	Pulmonary tuberculosis
2	4	0.15	" "
3	8	0.29	Chronic cardiac decomposition
4	9	0.12	Myocarditis
5	10	0.07	Cardiorenal

DISCUSSION

As shown in Table I (results on 20 typical fluids), the amount of organically bound phosphorus is very small, the maximum amount found in the examination of 200 fluids being 0.59 mg and the minimum 0.06 mg per cent. Only five fluids contained more than 0.4 mg per cent. Two of these showed a meningitic colloidal gold curve, one showed a luetic curve, one showed a negative colloidal gold curve, and one fluid was not recorded for this test.

The variation in absolute amount of organic phosphorus is very small and no relation to the pathology can be pointed out except that on the average those fluids containing more globulin and cells and which gave a positive Wassermann test showed most organic phosphorus. But even in this respect there were a number of exceptions.

Since practically no lipid phosphorus was found in the cerebrospinal fluid solids, the phosphorus is bound in some other form, the possibilities being, according to the present knowledge of phosphorus compounds of the body, a hexosephosphoric ester (Emden), a nucleotide (Jackson) or inosinic acid (Greenwald). At any rate, *it is impossible to point out any nerve-tissue changes by the amount of organic phosphorus.* This was the point in particular on which data were desired.

It appears from Table II that the change in the amount of organic phosphorus after death is very slow.

It can be seen from Table I that the inorganic and organic phosphorus are entirely independent of each other, e.g., fluids with high values for organic phosphorus may have high or low inorganic phosphorus content.

SUMMARY

Two hundred cerebrospinal fluids, from hospital and out patient cases, have been analyzed particularly as to content of organically bound phosphorus. Between 0.06 mg and 0.59 mg per cent of phosphorus was found, the great majority being between 0.1 mg and 0.3 mg per cent. This phosphorus is not in the form of lipoids but in all probability in protein combination.

No diagnostic value can yet be attached to the determination of organic phosphorus.

Organic and inorganic phosphorus contents are independent of each other.

Thanks are due Miss Marjorie Bauckus of the serologic laboratory for help in obtaining the fluids.

REFERENCES

- ¹Pincus, J. B., and Kramer, Ben. *Jour Biol Chem*, 1923, lvi, 463.
- ²Haurowitz, Felix. *Ztschr f physiol Chem*, 1923, cxxviii, 290.
- ³Cohen, Henry. *Quart. Jour Med.*, 1923, 24, xvii, 289.
- ⁴Hamilton, Bengt. *Jour Biol Chem*, 1925, lxx, 101.
- ⁵Mestrezat, W. *Le liquide cephalo rachidien*, 1912.
- ⁶Donath, J. *Arch f Psychiat*, 1907, xliii, 1356.
- ⁷Apelt, F., and Schumm, O. *Arch f Psychiat*, 1908, xlv, 845.
- ⁸Williamson, G. S. *Lancet*, 1909, i, 1047.
- ⁹Benedict, S. R., and Theis, Ruth C. *Jour Biol Chem*, 1924, lxx, 63.
- ¹⁰Bell, R. D., and Doisy, E. A. *Jour Biol Chem*, 1920, xlv, 55.
- ¹¹Youngburg, Guy E., and Pucher, Geo. W. *Jour Biol Chem*, 1924, lxx, 31.

The Edward N. Gibbs Memorial Prize Fund

The income of this fund, amounting to approximately \$1,000 annually for three years, is available for research upon diseases of the kidney.

Workers in properly equipped laboratories desiring to apply for a grant in this fund may make application to the Committee of the Edward N. Gibbs Memorial Prize Fund, The New York Academy of Medicine, Fifth Avenue and 103rd Street, New York City.

METHODS

Cultures of anhemolytic streptococci were obtained from eight sources. Five were from throat cultures of patients with rheumatic endocarditis, one was from a positive blood culture in the course of subacute bacterial endocarditis, two were obtained from throat cultures of normal individuals. Cultures from the throat were made on Loeffler's blood serum and also streaked on blood agar plates. Transplants from colonies of anhemolytic streptococci, usually green producing, were made in flasks of blood-dextrose bouillon and in bottles of Loeffler's media containing considerable condensation fluid. All the strains studied fermented lactose and salicin, but not mannitol. After incubation for two to three days, the sediment was removed by centrifugation, the supernatant fluid diluted with an equal volume of normal saline and filtered through Berkefeld N filters. Varying speeds of filtration were used but no apparent difference in toxicity due to this cause, was noted. The filtrates were used as inocula. Filtrates which had been heated for varying periods in the Arnold sterilizer and in the autoclave under 15 pounds pressure were also studied. Guinea pigs were inoculated by intramuscular and intra-peritoneal injections. Intracardiac methods were tried but found uncertain and not suitable because of the resulting interference with histologic examination.

PROTOCOLS OF RHEUMATIC FEVER PATIENTS

1 *Acute Rheumatic Fever*—H G, male, aged forty four, had mild attack of joint pains three years prior to the present observation, at which time he developed polyarthritis. Associated with this he had fever varying between 99° F and 101.6° F. The heart at no time was affected. The tonsils were diseased and cultures produced a profuse growth of *Streptococcus viridans*. Roentgenograms of the teeth revealed periapical infection.

2 *Rheumatic Endocarditis and Pericarditis*—E L, male, aged seventeen years. First attack of rheumatic fever three years before present observation at which time he developed endocarditis. Recurrence at present observation with fever, pericarditis and mitral presystolic and systolic murmurs. Heart enlarged to right and left with left border to the sixth rib in the anterior axillary line. P R interval 0.14 second.

3 *Subacute Bacterial Endocarditis*—H L, male, aged seventy two years. Chills and fever two months before admission, died one month after admission. Irregular, remittent fever, as high as 104° F while in hospital. Apex beat fifth interspace just outside mid clavicular line, a harsh mitral systolic murmur. Spleen was not palpable but liver was enlarged. No petechiae and no clubbing of fingers were noted. Blood count showed 2,910,000 red blood cells, hemoglobin 56 per cent, leucocytes 14,000. The urine showed a heavy cloud of albumin, red blood cells and granular casts. Blood cultures on two trials were positive for *Streptococcus viridans*. Necropsy revealed typical luxuriant and friable vegetations of the mitral valve extending up into the left auricle.

4 J F, male, aged thirty one, was admitted to hospital complaining of shifting pains and swelling in large joints of two weeks' duration. No previous attacks. Large diseased tonsils were present, a loud systolic murmur was heard at the mitral area transmitted to the axilla. Anhemolytic streptococci were found on throat culture. Temperature ranged between 98° F and 100° F. Electrocardiogram revealed widening of the P wave to 0.1 second with normal P R interval. Marked improvement was noted in seven weeks, the murmur was not heard at the final examination.

5 *Acute Rheumatic Fever and Endocarditis*—J G, boy, aged thirteen years. Onset with sore throat and fever followed one week later by arthritis of both knees, rough systolic murmur at aortic area and slight enlargement of heart to left.

6 *Rheumatic Fever and Endocarditis Following Tonsillitis*—J C, male, twenty six years of age, developed polyarthritis two weeks after an attack of tonsillitis. Ankles, knees, hips and shoulders were successively involved. Urticaria present. Diseased tonsils, enlargement of the heart and mitral presystolic and systolic murmurs were noted.

RESULTS

Thirty four guinea pigs averaging 300 gm. in weight, were injected with the Berkefeld filtrates of cultures of anhemolytic streptococci recovered from the throats of five patients with rheumatic fever and endocarditis with sterile blood cultures, and from the blood of a patient with subacute bacterial endocarditis. Intramuscular and intraperitoneal injections were made in doses of 2 to 5 cc (representing 1 to 25 cc of undiluted filtrate). Twenty three of the animals died in periods varying from one to twenty one days. Deterioration was noted in all the cultures studied and after a number of transplantations, the lethal effect was gradually lost. The details are more clearly brought out in the table. Attempts to modify the toxic principle by heat

TABLE I
EFFECT OF INJECTION OF STREPTOCOCCIC FILTRATES INTO GUINEA PIGS

ANIMAL NO	CULTURE	FILTRATE AMT CC*		METHOD OF INOCULATION	RESULT
		Unheated	Heated		
1	H G	3 cc		Intramuscular	Died 21 days
2	"	4 cc		"	Died 18 "
3	E L	2 cc		"	Died 14 "
4	"	4 cc		"	Died 12 "
5	"	3 cc		"	Died 16 "
6	"	2 cc		"	Survived
7	"	2 cc		"	Died 19 "
8	"	3 cc		"	Died 15 "
9	"	5 cc		Intraperitoneal	Died 5 "
10	"	5 cc		"	Died 11 "
11	"	4 cc		"	Died 19 "
12	"	4 cc plus 1 cc serum of E L		Intramuscular	Died 4 "
13	"	4 cc plus 1 cc control serum		"	Died 4 "
14	"		90 C 1 hr 3 cc	"	Died 9 "
15	"		100 C 1 hr 3 cc	"	Died 22 "
16	H L	1 cc		Intraperitoneal	Died 0 "
17	"	2 cc		"	Died 4 "
18	"	3 cc		"	Died 3 "
19	"	4 cc		"	Died 24 hours
20	"	3 cc		"	Died 13 days
21	"	5 cc		"	Died 10 "
22	"†	5 cc		"	Survived
23	"	5 cc		"	Survived
24	J F	2 cc		"	Survived
25	"	5 cc		"	Survived
26	J G	3 cc		"	Died 1 day
27	"	2 cc		"	Died 3 days
28	"	1 cc		"	Died 9 "
29	H T (normal)	1 cc		"	Survived
30	"	5 cc		"	Survived
31	E S (normal)	1 cc		"	Survived
32	"	5 cc		"	Survived
33	J C	4 cc		"	Survived
34	"	5 cc		"	Survived

Filtrate represents 1 dilution of supernatant fluid of culture media.

†Culture in fifth transplantation

A STUDY OF THE TOXIC FILTRATES OF ANHEMOLYTIC
STREPTOCOCCI, RECOVERED FROM PATIENTS
WITH RHEUMATIC FEVER*

BY EDWARD STEINFIELD, M D, AND MAURICE S JACOBS, M D
PHILADELPHIA, PA

THE literature of rheumatic fever contains numerous researches dealing with the relation of streptococci to this disease. Probably the most exhaustive is the well-known contribution of Poynton and Paine¹. Other investigators have endeavored to adduce evidence in favor of the streptococcal origin of rheumatic fever and rheumatic endocarditis, or have indicated such relationship by the demonstration of immune reactions in patients with this disease. Among the early workers, Triboulet and Coyon² implicated a diplococcus found in the blood of rheumatic fever patients, Westphal, Wassermann and Malkoff³ cultivated from the heart's blood in fatal cases, a streptococcus which grew in mediums of high alkalinity. Tunnichiff⁴ found that opsonins for streptococci were increased in the blood of patients with acute rheumatic fever and in seven out of twelve cases, agglutinins were demonstrated. Kinsella and Swift,⁵ however, were unable to demonstrate that strains of anhemolytic streptococci recovered from patients with rheumatic fever belonged in the same biochemical or immunologic group. Denick and Andrewes⁶ have noted differences between strains of anhemolytic streptococci derived from throat cultures of patients with rheumatic fever and cultures from normal individuals. The former gave skin reactions in rabbits with a secondary reaction nine days later, this did not occur with anhemolytic streptococci from normal throats. Interesting observations have been recorded by Miller⁷ in attempts to transmit rheumatic fever to rabbits and guinea pigs with various materials from patients with this disease, supposedly containing the infective agent. He used (1) blood (usually uncoagulated) taken from the vein during acute stages of rheumatic fever, (2) joint fluid aspirated from the involved joints and anaerobic cultures from the joint fluid, (3) pleural fluid, (4) throat washings which had been passed through a Berkefeld N filter, (5) extracts of tonsillar tissue. These inocula were injected into twenty seven young rabbits and fourteen young guinea pigs. A definite arthritis occurred in only one rabbit and one guinea pig. The guinea pig had been directly inoculated from the throat washings of a patient. The rabbit, however, had been injected with the blood and suspension of heart muscle from another rabbit which had received the whole blood from a patient with rheumatic fever. Though these two observations may be said to be isolated instances, they are suggestive, as Miller points out, because of the unlikelihood of spontaneous arthritis in rabbits and guinea pigs.

*From the Laboratories of the Jewish Hospital
Received for publication December 31 1926

Among the cogent arguments against the streptococcic etiology of rheumatic endocarditis and myocarditis is the inability to produce true Aschoff nodules in the animal after injection with anhemolytic streptococci. Topley and Weir⁸ inoculated twenty nine rabbits with cultures of streptococci isolated at necropsy from the mitral valve of a case of rheumatic endocarditis. Of these, twenty six developed fever, twenty three developed arthritis and two developed endocarditis. The infiltrations in the heart, however, resembled Bracht Wachter lesions rather than the characteristic submiliary nodules described by Aschoff. Thalheimer and Rothschild,⁹ and later Cecil¹⁰ described the distinguishing features in the hearts of animals injected with streptococci and indicated their resemblance to lesions of subacute bacterial endocarditis in contrast to those of rheumatic fever. The possible error due to infiltrations produced by foreign protein has been pointed out by Longcope¹¹ who described collections of round cells in the heart muscle of rabbits after injection of egg albumen and horse serum. Another factor has been noted by Miller¹ in the spontaneous interstitial myocarditis found in apparently normal rabbits.

Blood cultures taken in the course of rheumatic fever are frequently sterile in contradistinction to the high percentage of positive blood cultures in subacute bacterial endocarditis. Recently Clawson¹² has described observations in which strains of *Streptococcus viridans* were isolated from twenty cases of well defined acute rheumatic fever, rheumatic endocarditis or chorea of which thirteen were derived from blood cultures during life. The high percentage of positive blood cultures is attributed by him to the following technique. Fifty cc of blood were collected in two test tubes and allowed to clot, the clots are later loosened and put into flasks of 250 cc of dextrose beef infusion. Of twelve strains tested nine produced endocarditis in animals with apparently typical vegetations and the organisms were recovered in the heart's blood and joint. Agglutinins for the streptococci were found in four out of five cases of rheumatic fever in dilution of 1:50 or more.

Though our own studies are concerned with a toxic principle derived from cultures of anhemolytic streptococci, presumably an exotoxin, the work of Herry¹⁴ upon an endotoxin from similar strains is somewhat applicable. Since most of these strains, however, were obtained from blood cultures it suggests the possibility that some of these patients may have suffered from subacute bacterial endocarditis. After grinding sediments of these cultures with salt and resuspending in distilled water for twenty four hours he used the clear supernatant fluid filtered through a Chamberland filter. This material produced death in rabbits in five to twelve days. He described lesions in the heart muscle which were thought to resemble Aschoff bodies.

The present report is based upon the toxic effects of Berkeley filtrates from cultures of anhemolytic streptococci, derived from throat cultures of patients with rheumatic fever or rheumatic endocarditis. For the purpose of comparison similar strains from normal individuals and one strain isolated from blood cultures of a patient with subacute bacterial endocarditis were also used.

METHODS

Cultures of anhemolytic streptococci were obtained from eight sources. Five were from throat cultures of patients with rheumatic endocarditis, one was from a positive blood culture in the course of subacute bacterial endocarditis, two were obtained from throat cultures of normal individuals. Cultures from the throat were made on Loeffler's blood serum and also streaked on blood agar plates. Transplants from colonies of anhemolytic streptococci, usually green producing, were made in flasks of blood-dextrose bouillon and in bottles of Loeffler's media containing considerable condensation fluid. All the strains studied fermented lactose and salicin, but not mannitol. After incubation for two to three days, the sediment was removed by centrifugation, the supernatant fluid diluted with an equal volume of normal saline and filtered through Berkefeld N filters. Varying speeds of filtration were used but no apparent difference in toxicity due to this cause, was noted. The filtrates were used as inocula. Filtrates which had been heated for varying periods in the Arnold sterilizer and in the autoclave under 15 pounds pressure were also studied. Guinea pigs were inoculated by intramuscular and intraperitoneal injections. Intracardiac methods were tried but found uncertain and not suitable because of the resulting interference with histologic examination.

PROTOCOLS OF RHEUMATIC FEVER PATIENTS

1 *Acute Rheumatic Fever*—H G, male, aged forty four, had mild attack of joint pains three years prior to the present observation, at which time he developed polyarthritides. Associated with this he had fever varying between 99° F and 101.6° F. The heart at no time was affected. The tonsils were diseased and cultures produced a profuse growth of *Streptococcus viridans*. Roentgenograms of the teeth revealed periapical infection.

2 *Rheumatic Endocarditis and Pericarditis*—E L, male, aged seventeen years. First attack of rheumatic fever three years before present observation at which time he developed endocarditis. Recurrence at present observation with fever, pericarditis and mitral presystolic and systolic murmurs. Heart enlarged to right and left with left border to the sixth rib in the anterior axillary line. P R interval 0.14 second.

3 *Subacute Bacterial Endocarditis*—H L, male, aged seventy two years. Chills and fever two months before admission, died one month after admission. Irregular, remittent fever, as high as 104° F while in hospital. Apex beat fifth interspace just outside mid clavicular line, a harsh mitral systolic murmur. Spleen was not palpable but liver was enlarged. No petechiae and no clubbing of fingers were noted. Blood count showed 2,910,000 red blood cells, hemoglobin 56 per cent, leucocytes 14,000. The urine showed a heavy cloud of albumin, red blood cells and granular casts. Blood cultures on two trials were positive for *Streptococcus viridans*. Necropsy revealed typical luxuriant and friable vegetations of the mitral valve extending up into the left auricle.

4 J F, male, aged thirty one, was admitted to hospital complaining of shifting pains and swelling in large joints of two weeks' duration. No previous attacks. Large diseased tonsils were present, a loud systolic murmur was heard at the mitral area transmitted to the axilla. Anhemolytic streptococci were found on throat culture. Temperature ranged between 98° F and 100° F. Electrocardiogram revealed widening of the P wave to 0.1 second with normal P R interval. Marked improvement was noted in seven weeks, the murmur was not heard at the final examination.

5 *Acute Rheumatic Fever and Endocarditis*—J G, boy, aged thirteen years. Onset with sore throat and fever followed one week later by arthritis of both knees, rough systolic murmur at aortic area and slight enlargement of heart to left.

6 *Rheumatic Fever and Endocarditis Following Tonsillitis*—J C, male, twenty six years of age, developed polyarthrits two weeks after an attack of tonsillitis. Ankles, knees, hips and shoulders were successively involved. Urticaria present. Diseased tonsils, enlargement of the heart and mitral presystolic and systolic murmurs were noted.

RESULTS

Thirty four guinea pigs averaging 300 gm in weight, were injected with the Berkefeld filtrates of cultures of anhemolytic streptococci recovered from the throats of five patients with rheumatic fever and endocarditis with sterile blood cultures, and from the blood of a patient with subacute bacterial endocarditis. Intramuscular and intraperitoneal injections were made in doses of 2 to 5 cc (representing 1 to 25 cc of undiluted filtrate). Twenty three of the animals died in periods varying from one to twenty one days. Deterioration was noted in all the cultures studied and after a number of transplantations, the lethal effect was gradually lost. The details are more clearly brought out in the table. Attempts to modify the toxic principle by heat

TABLE I
EFFECT OF INJECTION OF STREPTOCOCCIC FILTRATES INTO GUINEA PIGS

ANIMAL NO	CULTURE	FILTRATE AMT CC*		METHOD OF INOCULATION	RESULT
		Unheated	Heated		
1	H G	3 cc		Intramuscular	Died 21 days
2	"	4 cc		"	Died 18 "
3	E L	3 cc		"	Died 14 "
4	"	4 cc		"	Died 12 "
5	"	3 cc		"	Died 16 "
6	"	2 cc		"	Survived
7	"	2 cc		"	Died 19 "
8	"	3 cc		"	Died 15 "
9	"	5 cc		Intraperitoneal	Died 5 "
10	"	5 cc		"	Died 11 "
11	"	4 cc		"	Died 19 "
12	"	4 cc plus 1 cc serum of E L		Intramuscular	Died 4 "
13	"	4 cc plus 1 cc. control serum		"	Died 4 "
14	"		90 C 1 hr 3 cc	"	Died 9 "
15	"		100 C 1 hr 3 cc	"	Died 22 "
16	H L	1 cc		Intraperitoneal	Died 6 "
17	"	2 cc		"	Died 4 "
18	"	3 cc		"	Died 3 "
19	"	4 cc		"	Died 24 hours
20	"	3 cc		"	Died 13 days
21	"	5 cc		"	Died 10 "
22	"†	5 cc		"	Survived
23	"	5 cc		"	Survived
24	J F	2 cc		"	Survived
25	"	5 cc		"	Survived
26	J G	3 cc		"	Died 1 day
27	"	2 cc		"	Died 3 days
28	"	1 cc		"	Died 9 "
29	H T (normal)	4 cc		"	Survived
30	"	5 cc		"	Survived
31	E S (normal)	4 cc		"	Survived
32	"	5 cc		"	Survived
33	I C	4 cc		"	Survived
34	"	5 cc		"	Survived

Filtrate represents 1 : dilution of supernatant fluid of culture media.
† Culture in fifth transplantation

less than 100° C, or by the addition of normal serum or serum from patients with rheumatic fever, appeared to enhance the toxicity. In all instances several days before death the heart rate of the animal was considerably lowered, frequently falling from a normal rate of 180 to 200 beats per minute down to 100 beats per minute, as noted by auscultation. A loss in weight was also observed if the animal survived over a week. No instance of arthritis was noted. At necropsy, cultures from the heart's blood were sterile. Firm clots were found in the heart cavity but no discernible lesion of the valves was noted, though obviously conclusions on gross inspection of these structures were invalidated by their small dimensions. Microscopic section and staining with hematoxylin and eosin revealed no areas resembling Aschoff bodies. Selective staining with the Unna-Pappenheim method was not done because nothing suggesting submiliary collection of plasma cells was noted. A slight cloudy swelling of the myocardium was found in some of the specimens. The toxic principle was apparently not produced regularly by all strains found in throat cultures from rheumatic patients. In similar attempts in two other cases, entirely negative results were obtained. Negative results were also noted with the strains from two normal individuals.

DISCUSSION

The question as to whether we were dealing with a true exotoxin is not definitely determined. Experiments in producing an antitoxin capable of protecting the animal in multiple proportions, were only partially carried out due to the large doses of filtrate necessary to establish this point. The toxic property is rather small when the comparatively large dose is considered. Its marked resistance to heat creates a resemblance to an endotoxin though the same property is manifested by Dick's scarlatinal toxin. The definite though variable incubation period regularly observed is however more often noted with a true exotoxin. The experiments of Herry indicated similar incubation periods with the use of material presumably containing an endotoxin.

SUMMARY

1 Berkefeld filtrates of cultures of anhemolytic streptococci isolated from the throats (particularly the tonsils) of three patients with rheumatic fever were lethal for guinea pigs in doses of 2 to 4 c c (representing 1 to 2 c c of original culture fluid). This property was also noted in the cultures of anhemolytic streptococci isolated from the blood of a patient with subacute bacterial endocarditis. Two other strains isolated from patients with rheumatic fever and two from normal individuals did not produce toxic filtrates.

2 The action of the toxic filtrate was evidenced by a slowing of the heart rate in guinea pigs and death in one to two weeks with some strains and in one to nine days with other strains. Deterioration was noted after a number of transplants with a loss in lethal power. Aschoff nodules and arthritis were not produced in the experimental animals.

3 The toxic principle, when present, was relatively weak in view of the comparatively large doses which were used.

REFERENCES

- ¹Poynton F J, and Paine A. *Researches on Rheumatism*, London, 1913 J A Churchill
- ²Triboulet and Croya. *Bull et Mem Soc Méd d hop de Paris*, 1898, p 93
- ³Westphal, Wassermann and Malkoff. *Berl klin Wehsehr*, 1899, *xxx*, 638
- ⁴Tuanichiff R. *Jour Infect Dis*, 1909, *vi*, 346
- ⁵Kinsella, E A., and Swift, H F. *Arch Int Med*, 1917, *xix*, 381
- ⁶Derick, C L., and Andrewes, C H. *Jour Exper Med* July 1926, *xliv*, 35
- ⁷Miller, C P. Jr. *Jour Exper Med* October, 1924, *xl*, 525
- ⁸Topley, W W C., and Weir, H B. *Jour Path and Bacteriol* 1921, *xxiv*, 333
- ⁹Thalhimer, W., and Rothschild, M A. *Jour Exper Med*, 1914, *xix*, 429
- ¹⁰Cecil, R L. *Jour Exp Med*, 1916, *xxiv* 739
- ¹¹Longcope, W T. *Arch Int Med* 1915 *xv* 1079
- ¹²Miller, C P., Jr. *Jour Exper Med*, 1924, *xl* 543
- ¹³Clawson, B J. *Jour Infect Dis* 1925 *xxxvi* 445
- ¹⁴Herry. *Bull Acad roy Med Belg* 1914 *xxviii* 76 (series 4)

THE ENDOTHELIOID CELL IN ACUTE LEUCEMIA*

B. R. B. BARTON M.D., BOSTON MASS

THE significance of endothelial and endothelioid cells in the leucemias has been the basis of considerable speculation with very little substantial experimental background. It is known that in chronic myeloid leucemia the absolute endothelial count is increased, this usually being ascribed to a concomitant reaction of the endothelial system to the leucemic stimulus. Further, a third classification of leucemias—the endothelial cell leucemias,—has been made. The latter, as reported, have all been more or less acute in course, clinically resembling acute leucemia of either conventional type.

Undoubtedly, the so called endothelial cell leucemia is a rare condition, although in all probability not so rare as the literature indicates. The cases that have been reported are noteworthy for the paucity of pathologic detail they present with but one outstanding exception. Ewald† has reported a case of acute leucemia, with a rapidly fatal course in which the predominant cells were distinctly endothelioid,—similar, indeed in every respect to those seen in the case outlined below. He prepared excellent sections which showed definite extracapillary budding of the atypical cell within the bone marrow. This would seem to give it a place in the leucocyte series disregarding the obvious morphologic evidence, in fact, Ewald hinted that it might be an earlier form of myeloblast although he eventually classified it as erythroblast. The Ewald case and the extraordinarily fine histopathologic preparations for which Ewald is responsible go far toward substantiation of the conclusions drawn from the following case.

CASE HISTORY

On February 16, 1926 a man thirty six years old was admitted to the Haynes Memorial Hospital complaining of asthenia and vertigo of about ten days' duration. The referring physician suspected diphtheria as there was a heavy yellow white membrane on the hard palate and the left tonsil. There were marked gastrointestinal symptoms—nausea vomiting and diarrhea with tarry stools. The patient was obviously very ill being dyspneic and cyanotic,

*From the Evans Memorial.

†Ewald Otto. *Deutsches Arch f klin Med* 19 *cxiii*

and tossing restlessly in bed. His lips were pale, the mouth dry and incrustated with sordes, and the facial color was gray with a distinctly yellowish cast. Examination revealed little further. The heart was slightly enlarged, and there were loud murmurs of the hemic type over all the valve areas. The abdomen was negative. A neurologic examination, which was done when a question of pernicious anemia arose, was essentially negative. There was no demonstrable adenopathy, and the spleen was not palpable. The patient remained in the hospital seven days with a temperature oscillating between 101.2 and 102.4, and died on the morning of the eighth day with pulmonary edema and myocardial failure. The only therapy given consisted of a prophylactic dose of 30,000 units of diphtheria antitoxin on admission, and a liquid diet. Smears prepared from the membrane on the tonsil showed a definite Vincent's angina. Necropsy was performed about five hours postmortem.

Gross examination of the tissues showed nothing more than a pulmonary edema and dilated heart. The cardiac tissue was of the "tiger lily" type, showing marked fatty degeneration. There was no adenopathy beyond a very slight enlargement of the mesenteric glands, which were removed for section. Microscopic examination of spleen, liver, bone marrow and lymph nodes showed typical leucemic infiltration in which the atypical cell of the circulating blood was predominant. The bone marrow, which was pink and liquescent in the gross, was taken from the shaft of the femur. Unfortunately, it was not possible to demonstrate the direct proliferation of the cells from the bone marrow capillaries in a satisfactory manner.

The day following admission of the patient a blood count was done by the interne. A red count of 1,370,000 and a white of 2,000 was reported. The differential count showed 80 per cent of "lymphoid" cells. Daily counts thereafter showed a tendency to lowering of the white count, which dropped to 1,300 on the third day. On February 27, the blood was examined in the Evans Memorial laboratories, the question of pernicious anemia having arisen. At this time a red count of 1,350,000 and a leucocyte count of 3,650, of which 57.8 per cent were myeloblasts, was obtained. The myeloblasts were entirely characteristic, most of them yielding a peroxidase reaction with the Goodpasture stain. The following morning, five hours before the patient's death, a second count was made. The red count was very slightly increased, while the leucocyte count had risen to a distinctly leucemic value of 29,950. Coincident with this rather remarkable change, the character of the predominant cell was altered, the myeloblasts which here formed only 23.5 per cent of the differential count, having been replaced by a cell classified as endotheloid which formed 46 per cent of the total count.

The endotheloid cell, which could not be sharply differentiated from the myeloblast in all cases, showed the following characteristics. The diameter varied from 10 to 40 microns, the average being about 20 microns. The outline was irregular, in some cases showing a tendency toward separation of cytoplasmic fragments, strongly suggestive of the megakaryocyte. The nucleus was multilobulated, presenting a fine granular appearance characteristic of many early cells. There were present from one to four nucleoli or plasmosomes. In not a few instances the cell was polynuclear. The cytoplasm stained deeply basophilic and somewhat unevenly, a perinuclear zone being almost unstained with both Wright's and Giemsa's stains. The granules were irregular in size, azurophilic to slightly basophilic and showed a tendency to grouping and peripheral distribution. The cells in many instances yielded a marked peroxidase granulation with the Goodpasture stain. As has been intimated, the morphology varied considerably, ranging from a type undoubtedly endothelial to one distinctly myeloblastic. The leucemia was complicated by a marked anemia with many primary characteristics and a tendency to aplasia.

It is believed that the case above should be diagnosed as a premyeloblastic or endotheloid, rather than an endothelial cell leukemia as had been suggested. The evidence to support this contention is found both in the case reported above and in the Ewald case. The case described, on admission was distinctly that of aleucemic leukemia evidently of a rapidly progressive type. This was accompanied by an anemia which was somewhat aplastic. In view of these two conditions the bone marrow may be said to have become exhausted or to have lost its power to a large extent, of both erythro- and leucogenesis. A few

TABLE I
BLOOD COUNTS

SEPT 22		SEPT 23	
Erythrocytes	1,350 000	1 550,000	
Leucocytes	3,050	29,950	
Hemoglobin	30%	35%	
Color index	1.1	1.1	
Platelets	130 000	230 000	
Differential Count			
Cells counted	500		200
Lymphocytes, small	18%	16.5	
large	4.6%	0.5	
Plasma cells	0.2		1.5
Mast "	0.2		0
Eosinophiles	0		0
Polymorphonuclears	5.4		5.5
Endothelial	1 mono 0.8 trans 2.6	3.4	0
Lymphoblasts	0.3		0
Basophilic myelocytes	0		0
Acidophilic "	0		0
Neutrophilic	4.8		1.5
Myeloblasts	57.8		45.5
Endotheloid cells	0.6		49.0
Megakaryocytes	0.6		0
Erythroblasts	0		0.5
In Above Differential Counts			
Anisocytosis	Marked, many megalocytes in both		
Poikilocytosis	Marked "primary" type in both		
Polychromasia	Definite		
Normoblasts	3		7
	No micro or megaloblasts		

hours before death the leucemia changed to the leucemic form, thus the-
retically thrusting upon the exhausted bone marrow already incapable of
producing more than an extremely immature cell, a tremendous strain. This
is believed to have provoked the production of a cell earlier in the myeloge-
nous series than the myeloblasts and in all probability the parent cell of
the myeloblast. It is also possible that the endotheloid cell represents an
abortive form not identical with any of the developmental forms in a myeloid
series. In any event, it indubitably represents an earlier form than has been
hitherto recognized. To substantiate these deductions Ewald's demonstration
of the direct extracapillary proliferation of the endothelial cells of the bone
marrow capillaries seems to give the lacking histopathologic evidence.

The findings above are not entirely unique. The endotheloid cell has
been further observed in far smaller numbers in two cases of rapidly fatal
acute myelogenous leucemia, and at the height of an exacerbation of a chronic
myelogenous leucemia in which myeloblasts were numerous. It is suggested
that it may yield some prognostic significance.

In conclusion, then, there is both direct and indirect evidence that there
is present in the circulating blood under certain unusual conditions a cell with
distinctly endothelial characteristics intermediate between the endothelial cell
of the bone marrow capillaries and the myeloblast of acute leucemias.

smears The complement-fixation test (Kolmer) was positive in all patients having syphilis The presence of brain tumor was proved at necropsy The other diagnoses were submitted by the clinical consultants

RESULTS

The results of this study are shown in Tables II to VII Table II contains a group of thirty-three apparently normal control specimens consisting chiefly of spinal fluids taken from patients under treatment for syphilis and from patients with other diseases probably not involving the central nervous system The range of the sugar content is from 60 to 90 mg These figures closely parallel those of Stowe, Spurling and Maddock and are much higher than the earlier reports by the French workers and by Foster

TABLE II
NORMAL CONTROLS

CASES	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, GRADE	SUGAR MG	GUINEA PIG INOCULATION
1	Suspected syphilis of the central nervous system	0	0	77	Negative
2	Pulmonary tuberculosis	0	0	70	
3	Sinusitis	0	2+	66	
4	Carcinoma of prostate	0	0	70	
5	Mercurial poisoning	6	0	75	
6	Pneumonia	0	1+	83	
7	Otitis media	0	0	68	
8	Syphilis	0	0	90	
9	Syphilis	0	0	80	
10	Syphilis	0	0	80	
11	Syphilis	0	0	75	Negative
12	Syphilis	0	0	72	
13	Syphilis	0	0	71	
14	Syphilis	0	0	68	
15	Syphilis	0	1+	81	
16	Syphilis	0	0	77	
17	Syphilis	0	0	70	
18	Syphilis	0	0	73	
19	Syphilis	0	0	82	
20	Syphilis	0	0	65	Negative
21	Syphilis	0	0	66	
22	Syphilis	0	0	73	Negative
23	Syphilis	0	0	72	
24	Syphilis	0	0	71	
25	Syphilis	0	0	82	
26	Syphilis	0	0	65	
27	Syphilis	0	0	72	Negative
28	Syphilis	0	0	74	
29	Syphilis	0	0	67	
30	Syphilis	5	0	79	Negative
31	Syphilis	0	0	73	
32	Syphilis	0	1+	60	
33	Syphilis	0	0	78	

The group including the various neurologic conditions (Table III), is of significance since the range of the sugar content is between 47 and 136 mg with an average of 107 mg Cases 2, 3 and 4 technically should be classified as encephalitis if the term is used in the general rather than in the restricted sense to cover cases of epidemic encephalitis lethargica Case 11 is of particular in-

THE DIAGNOSTIC VALUE OF THE SUGAR CONTENT IN THE CEREBROSPINAL FLUID*

BY ALFRED S. GIORDANO, M.D., M.Sc., SOUTH BEND, INDIANA†

THE intensive study during the last few years of the chemical composition of the cerebrospinal fluid has yielded a great deal of valuable information to aid in the differential diagnosis of certain obscure cases of brain tumor, encephalitis, poliomyelitis and various forms of meningitis. It is my purpose in this report to emphasize the value and the limitation of the quantitative determination of sugar in the differential diagnosis of such conditions with special reference to tuberculous meningitis.

In cases of pyogenic meningitis and tuberculous meningitis the sugar content of the cerebrospinal fluid is either zero or markedly diminished. In cases of poliomyelitis, encephalitis and other nonpurulent affections of the central nervous system the glucose content is extremely variable (Table I). Some authors, notably Foster and Cookson, have advanced the theory that the sugar content of the spinal fluid in encephalitis is relatively higher than in poliomyelitis. This conclusion is not generally accepted. At present there still exists some confusion as to the range of sugar in the normal spinal fluid.

I shall report additional data on this question based on a series of 127 examinations of spinal fluid, thirty-one were made in twenty-one cases of tuberculous meningitis and the remaining comprise a group of various normal and pathologic controls.

METHOD

The Folin and Wu method, with precipitation of the proteins by the tungstic acid reagents, was used for the quantitative sugar determinations. When the sugar content was below 25 mg. double amounts of filtrate were used or the dilution of the unknown was made up to 12.5 c.c. instead of 25 c.c., thus facilitating the reading. All nonpyogenic spinal fluids with a sugar content of less than 70 mg. were inoculated into guinea pigs to determine the presence or absence of bacilli of tuberculosis. All globulin estimations were made by the Noguchi method using 0.2, 0.5 and 0.1 c.c.

DIAGNOSIS

The diagnosis of tuberculous meningitis in all cases reported was corroborated either by necropsy, by guinea pig inoculation of the spinal fluid or by both, and in a few instances the bacilli of tuberculosis were demonstrated in smears. This method, however, was not depended on for obvious reasons. In the cases of pyogenic meningitis the diagnosis was proved by cultures and

*Read before the resident and ex-resident physicians of Mayo Clinic October 1926

†From the South Bend Medical Laboratory

Received for publication January 29 1927

TABLE III
VARIOUS NEUROLOGIC AFFECTIONS

CASES	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, GRADE	SUGAR MG	GUINEA PIG INOCULATION
1	Brain abscess	0	0	71.8	
2	Acidosis (acute enteritis)	20	4+	109.0	
3	Uremia (nephritis)	6	1+	136.0	
4	Meningism (pyelitis)	0	0	110.0	
5	Meningism (typhoid fever)	4	0	71.0	
6	Meningism (enteritis)	5	1+	70.6	
7	Meningism (enteritis)	1	0	60.5	Negative
8	Cerebral hemorrhage	30	1+	61.0	Negative
9	Cerebral hemorrhage	0	0	80.0	
10	Cerebral hemorrhage	*	1+	90.0	
11	Cerebral hemorrhage	3170 150**	1+	52.0	Negative
12	Intracranial hemorrhage	22	4+	80.0	Negative
13	Brain tumor	0	0	65.0	
14	Brain tumor	5	0	72.0	
15	Tetanus	3	0	77.0	
16	Tetanus	0	0	83.0	
17	Traumatic injury of head	0	0	90.0	
18	Epilepsy	0	0	70.0	
19	Epilepsy	6	0	86.0	
20	Psychoneurosis	0	0	86.0	
21	Psychoneurosis	0	0	88.0	
22	Manic depression	0	0	90.0	
23	Hydrocephalus	40	4+	75.0	
24	Neuritis	160	4+	106.0	

* Red blood cells

* Four days later

TABLE IV
SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

CASES	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN GRADE	SUGAR MG	WASSERMANN TEST (KOLMER)
1	Gastro crisis	50	2+	82	4, 4, 3, -
2	Tabes dorsalis	20	1+	70	4, 4, 4, -
3	Tabes dorsalis		2+	75	4, 4, 4, -
4	Charcot joint	130	4+	50	4, 4, 4, -
5	Juvenile tabes	60	3+	83	4, 4, 4, 4
		20	3+	52	4, 4, 4, 4
6	Paresis	77	-	66	4, 3, 1, -
7	Tabes dorsalis	10	1+	66	4, 4, -, -
8	Paresis	25	3+	60	4, 4, 4, -
9	Paresis	68	4+	74	4, 4, 4, -
10	Paresis	8	2+	67	4, 4, 4, 4
11	Tabes dorsalis	45	4+	63	4, 4, 4, -
12	Syphilis (treated)	8	1+	70	2, 2, 1, -
13	Wrist drop	35	2+	75	2, 2, 2, -
14	Gastro crisis	25	1+	85	2, 1, -, -
15	Tabes dorsalis	80	1+	65	4, 4, 4, 3
16	Tabes dorsalis	61	1+	88	4, 4, -, -
17	Tabes dorsalis	110	4+	58	4, 4, 4, 3
18	Suspected syphilis	100	1+	71	4, 4, -, -
19	Paresis	10	1+	70	2, 1, -, -

terest since the sugar content is within the range of tuberculous meningitis it also has all the other characteristics clear spinal fluid with a high cell count (3,170 for each cmm.), 1+ globulin, negative culture and no bacteria demon

TABLE V
PUJULENT MENINGITIS

CASE	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, OPADE	SUGAR, MG	ORGANISM FOUND ON CULTURE	ORGANISM FOUND IN SMEAR
1	Scarlet fever meningitis	350	4+	40	Streptococcus hemolyticus	Streptococcus
2	Influenza meningitis	2400	4+	0	None	Bacillus influenzae
3	Ulcerative endocarditis	700	4+	15	Streptococcus viridans	Streptococcus
4	Infected hydrocephalus	170	4+	35	Staphylococcus	Staphylococcus
5	Epidemic meningitis	2400	4+	5	Gram negative diplococcus	Meningococcus
6	Pneumococcal meningitis	2000	4+	0	Pneumococcus	Pneumococcus
7	Otitis media meningitis	1200	3+	0	Streptococcus viridans	Streptococcus
8	Otitis media meningitis	4000	4+	0	Streptococcus hemolyticus	Streptococcus
9	Meningitis	2300	4+	10	Streptococcus pyogenes	Streptococcus
1	Poliomyelitis	92	3+	62	None	None
2	Poliomyelitis	94	2+	94	None	None
3	Poliomyelitis	22	4+	77	None	None
4	Encephalitis	0	1+	81	None	None
5	Encephalitis	15	-	84	None	None
6	Encephalitis	0	2+	110	None	None
7	Encephalitis	0	0	90	None	None
		15*	0	183	None	None

*One hour before death

strable in the smear. The clinical findings, however, supported the presumptive diagnosis of cerebral hemorrhage or thrombosis and were further corroborated by the subsequent improvement of the patient and the negative result of the guinea pig inoculation for bacilli of tuberculosis. This is the only instance in this series of cases in which a diagnosis of tuberculous meningitis based on the low sugar content would have been erroneous. But is it sufficient to demonstrate the need of additional data on the subject?

The group of patients having neurosyphilis (Table IV) is easily distinguished from those having meningitis because of the positive Wassermann test, the sugar content is well within the normal range. The small group having purulent meningitis (Table V) shows absence of sugar or very low sugar con-

TABLE VI
TUBERCULOUS MENINGITIS

CASE	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN GRADE	SUGAR MG	SMEAR FOR BACILLI OF TUBERCULOSIS	GUINEA PIG INOCULATION	REMARKS
1	Tuberculous meningitis (four days later)	165	1+	28	-	+	Necropsy
2	Undetermined	98	1+	22	-	+	Necropsy
3	Poliomyelitis	33	3+	0	-	+	
4	Puerperal sepsis	248	4+	10	-	+	
	(two days later)	450	4+	37	-	+	
	(five days later)	430	4+	23	-	+	
		320	4+	2	-	+	Necropsy
5	Tuberculous meningitis	500	4+	5	+	+	
6	Tuberculous meningitis	101	4+	0	-	+	
7	Undetermined	278	2+	37	+	+	
8	Meningitis	43	1+	40	-	+	
9	Tuberculous meningitis	120	2+	0	+	+	
10	Pulmonary tuberculosis	6	1+	31	-	+	Necropsy
11	Encephalitis	192	1+	18	-	+	
12	Brain abscess	180	4+	3	+	+	
13	Basal meningitis (five days later)	97	3+	60	-	+	
	(ten days later)	230	2+	55	-	+	
		230	3+	43	+	+	
14	Subacute endocarditis (postmortem)	18	1+	5	-	+	Necropsy
15	Influenza	400	2+	11	-	+	
		300	2+	13	-	+	
16	Pyloric stenosis (three days later)	70	1+	60	-	+	Necropsy
	(eleven days later)	90	3+	48	-	+	
		90	2+	31	-	+	
17	Encephalitis	42	1+	25	+	+	Necropsy
18	Poliomyelitis	45	2+	0	-	+	
19	Meningitis	140	2+	25	-	+	
20	Tuberculous meningitis	110	3+	19	-	+	
21	Tuberculous meningitis	65	3+	26	-	+	
		190	3+	8	+	+	Necropsy

All cultures negative

tent and is differentiated from those having tuberculous meningitis by the demonstration of the causative bacteria either by smear or culture.

Cases of poliomyelitis and epidemic encephalitis (Table V) are placed in one group, the sugar content shows no differential variations.

There were twenty-one proved cases in the group diagnosed tuberculous meningitis (Table VI). The sugar content ranged from 0 to 60 mg. This figure is higher than has been reported. This may be due to the fact, however, that in

Cases 13 and 16 the lumbar puncture was done five to ten days before any clinical signs of meningeal irritation became apparent, but as the disease progressed the sugar content gradually fell to a low level. It is of particular interest to note the percentage of error in the clinical diagnosis on the admission of these patients to the hospital, but in most instances the clinical findings were too indefinite to diagnose them otherwise.

SUMMARY AND CONCLUSIONS

The data collected in this study corroborate recent studies on the sugar content of the spinal fluid. The normal range is probably between 60 to 90 mg for each 100 cc of fluid. The present data offer no differential points between poliomyelitis, encephalitis, brain tumor and other nonbacterial affections of the central nervous system. The sugar content, however, easily distinguishes them from meningitis. While the sugar content in cases of tuberculous meningitis may be in the same range as in case of purulent meningitis, it is generally higher and seldom zero. The distinguishing point, however, between these two important groups lies in the demonstration of the causative organism either by culture or smear and this is usually easily accomplished in the cases of purulent meningitis.

In cases of tuberculous meningitis the spinal fluid is usually clear and the cells are mostly lymphocytes while in the presence of purulent meningitis the fluid is usually cloudy and the polymorphonuclear leucocytes are the predominating cells. The cell count in cases of tuberculous meningitis is seldom higher than 500, while in cases of purulent meningitis it is seldom lower than 500. In my series it has been shown that the spinal fluid yields diagnostic data long before definite clinical signs of meningeal irritation become apparent and when these are added to the clinical history the diagnosis is definite.

The sugar concentration of the cerebrospinal fluid is of valuable diagnostic significance in the differential diagnosis of diseases involving the central nervous system.

REFERENCES

- ¹Cooksou, H. A. Diagnostic Value of the Sugar Content of the Cerebrospinal Fluid in Encephalitis Lethargica, *Brit Med Jour*, 1925, 1, 157.
- ²Folin, Otto, and Wu, Hsien. A System of Blood Analysis, *Jour Biol Chem*, 1919, **xxvii**, 81-110.
- ³Foster, H. E. Hyperglycemia in Epidemic Encephalitis, *Jour Am Med Assn*, 1921, **lxvi**, 1300-1301.
- ⁴Foster, H. E., and Cockrell, J. R. Cerebrospinal Fluid in Encephalitis Lethargica, *Am Jour Med Sc*, 1924, **clviii**, 696-702.
- ⁵Kubic, L. S., and Shults, Gertrude, M. Studies on the Relationship of the Chemical Constituents of Blood and Cerebrospinal Fluid, *Jour Exper Med*, 1925, **xl**, 565-591.
- ⁶Levinson, A. Comparative Sugar Content of Blood and of Cerebrospinal Fluid in Various Conditions, *Am Jour Dis Child*, 1925, **xxx**, 774-779.
- ⁷McLain, S. L., and Von Hofe, F. H. Further Studies of Cerebrospinal Fluid in Infants and Young Children, *Am Jour Med Sc*, 1925, **clxx**, 82-98.
- ⁸Spurling, R. G., and Maddock, C. L. The Cerebrospinal Fluid in Tumor of the Brain, *Arch Neurol and Psychiat*, 1925, **xv**, 54-63.
- ⁹Stowe, W. P. The Diagnostic Value of the Cerebrospinal Fluid Sugar Content, *Jour Lab and Clin Med*, 1926, **vi**, 307-312.
- ¹⁰Wilcox, H. B., and Lytle, J. D. The Diagnostic Value of Sugar Concentration in Spinal Fluid, *Arch Pediat*, 1923, **xl**, 215-225.
- ¹¹Wilcox, H. B., Lytle, J. D., and Hearn, J. E. The Chemical Composition of the Spinal Fluid, Diagnostic Value, *Am Jour Dis Child*, 1925, **xxx**, 513-540.

THREE PHASES OF WASSERMANN TECHNIC ASSOCIATED CONSIDERATIONS A COMPARISON OF ANTIGENS, AND A METHOD OF TITRATING AND ESTIMATING POSITIVITY OF A SERUM*

By MARY H. SWIN, M.D. CHICAGO, ILL.

PART I

THERE are three phases of Wassermann work which we wish to discuss briefly. First, associated considerations; secondly, antigens; and thirdly, a method of titrating positivity of a serum.

Of associated considerations there are a number of factors that are deserving of some emphasis and can well be frequently reiterated.

The care of glassware is of first importance. The glassware used for Wassermann work should be kept by itself and used for that department only. It is well to soak new material overnight in 2 per cent hydrochloric acid, but it should be most thoroughly rinsed afterward and allowed to stand overnight in water, and then rinsed again before drying for use.

In the routine cleaning of glassware acid preparations are not desirable and no form of soap need be used if care is taken. Pipettes can be placed in a tall jar of distilled water immediately after use. The distilled water is rather better than tap water in keeping away the greasy film which tends to form in the lumen of the pipette. The Wassermann tubes should be emptied as soon as the test is completed, rinsed out and filled with water. Then they can stand until a convenient time for cleaning. The tubes can be perfectly cleaned by using plain hot water and a stiff rod with a cotton swab on the end. The cotton can be frequently changed where there are many tubes. The inside of the tubes should be thoroughly rubbed, then rinsed and drained and put to dry in the oven. A finer wire with a bit of cotton is convenient for cleaning the inside of the pipettes. After washing they may be finally rinsed in distilled water.

It is desirable to use plenty of pipettes and a fresh one for every serum.

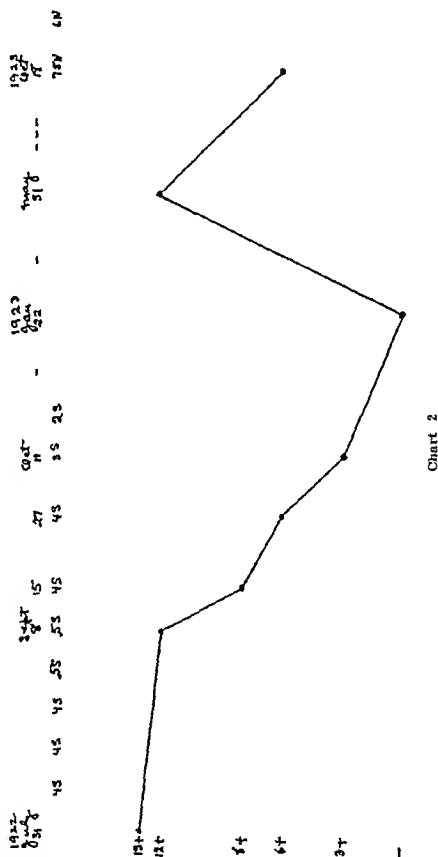
The next point is in regard to complement. Uniform diet for the guinea pigs such as oats, hay, carrots, some greens as lettuce or dandelions if they are available, helps to make satisfactory complement. The food given the guinea pigs must be in good condition. Partially decayed greens or carrots will make them sick. The guinea pigs should have no food the day they are bled for complement. Complement taken from a guinea pig with a full stomach often is low in activity and frequently gives a cloudy end point in titration. Several tubes will not clear quite completely, and as a result an

Read before the Illinois Medical Laboratory Association at Quincy, Illinois, May 19, 1906.
Received for publication Nov. 16, 1906.

From the Serologic Department of the National Pathological Laboratory, Chicago, Ill.

The value and custom of the fifteen minute inactivating period needs to appear often in print so that it may be very generally recognized as the proper procedure

We have done a good many Wassermann tests on serums in which the



native amboceptor was absorbed in one portion and not absorbed in the other. We prefer the results obtained when the native amboceptor is absorbed as a routine measure. The Kahn¹ method is very simple and effective. A drop of the washed cells is added per c.c. of serum. The tube is shaken, allowed to stand ten minutes at room temperature, then centrifuged.

The last point of associated considerations to be considered has to do with amboceptor and corpuscles

When corpuscles are obtained from an abattoir it may happen that a supply may be obtained which is extremely difficult to hemolyze. When the complement is titrated the result is very poor, too much complement being required, and the trouble may appear to be the complement when in reality it is the corpuscles. Fresh corpuscles should be obtained.

A $2\frac{1}{2}$ per cent suspension of corpuscles, just half the original 5 per cent suspension, is a very satisfactory percentage. We prefer the $2\frac{1}{2}$ per cent to the 2 per cent recommended by Dr. Kolmer in his test because the extra $\frac{1}{2}$ per cent gives just a little more color and body to the mixture. If one sensi-

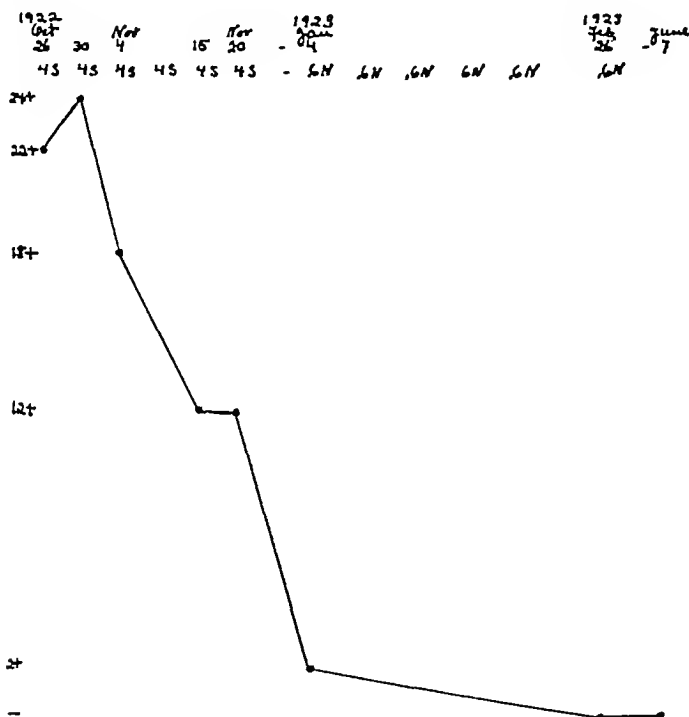


Chart 3—Patient B had a sore throat of several weeks duration and the eruption of secondary syphilis when the Wassermann was done Oct. 26 1922. The test was strongly positive with a titration of 22-plus. A series of six injections with German arsphenamine was given with 0.4 gm dosage beginning Oct 26. The patient had severe reactions from the salvarsan and the treatments were discontinued between Nov 20 and Jan 4. Beginning Jan 4 1923 a course of neosalvarsan dosage 0.6 gm was given at weekly or ten day intervals until Feb 26. The Wassermann was negative on that date. After the neosalvarsan a series of about ten mercurasol injections was given. From the beginning the patient took protiodide of mercury by mouth.

The curve of titration is shown in Chart 3.

izes the cells it is necessary to add all the corpuscles that are to be used at once to the given volume of amboceptor and mix thoroughly. For instance, if one is going to add 100 c.c. of corpuscles to 100 c.c. of amboceptor and instead of doing it all at once, pours 50 c.c. into the amboceptor, is then called to the telephone, returns and adds 50 c.c. more, the first 50 c.c. will absorb most of the amboceptor and there will not be much left for the next 50 c.c. The result will be a mixture carrying dead wood. When it is added to the

Wassermann tubes, and they come to the reading, there are apt to be false positives and false anticomplementary results. If the native amboceptor has not been previously absorbed in the serum some tubes will have enough perhaps to take care of the extra cells and some may not and there may be irregular results. For an example of this a technician once came to me who had been having trouble with his Wassermans because he had been adding his corpuscles a few at a time to the amboceptor. If the cells are not sensitized and the amboceptor is first added and then the corpuscles the tubes should be shaken immediately so that the corpuscles may be evenly loaded with the amboceptor and unbound complement. When the sensitized cells are prepared, a half hour at room temperature is sufficient time for the mixture to stand before use.

PART II

Because of the important place that antigens hold in the Wassermann test we are presenting the result of some work with various antigens in two sets of five thousand cases each.

First, we wished to observe how Dr Kolmer's antigen as prepared in his own laboratory, compared with the usual other three antigens, the cholesterolized, the plain alcoholic and the acetone insoluble as employed in the four hour ice box fixation.

Secondly, we desired to compare a group of three antigens prepared by Dr Kolmer's method, but without the use of the shaking machine with the three types of antigens of the first set.

The Kolmer antigens of the second group were prepared after Dr Kolmer's technique with the exception that neither the Soxhlet nor the mechanical shaker were used. One was made from Difco heart muscle powder, the other two from a mixture of three dried beef hearts for each antigen.

While our experience shows that all antigens fix to a somewhat greater degree in the eighteen hour ice box fixation this work was a comparison of antigens with the four hour fixation.

The proportions used in the test were one half the original Wassermann quantities as follows:

Total volume 20 cc
 Serum 0.1 cc and 0.05 cc portions in test and 0.1 cc and 0.1 cc in serum control tubes
 Salt 0.5 cc
 Antigens, complement, amboceptor and corpuscles so diluted as to use 0.5 cc each
 Sera were inactivated fifteen minutes at 55°C
 The native amboceptor was absorbed by adding one drop of washed sheep cells to each 1 cc of serum, allowed to stand not more than ten minutes at room temperature and then centrifuged.

The antigens in the first series were 0.1 per cent cholesterolized antigen. Dr Kolmer's antigen prepared in his laboratory, plain alcoholic antigen made similar to the Neymann Gager's method, and the acetone insoluble preparation. Those used in the second series were the same type as the first with the exception that the Kolmer antigen was not prepared in his own laboratory but as previously stated. The titration of antigens was similar to the Kolmer's method and ten units were used in the test. Four antigen control

tubes were used with two, three, four and five times the strength employed in the test

Complement was pooled from three guinea pigs. It was titrated by using 1 to 20 dilution, beginning with 0.08 c c and increasing 0.02 c c each tube as 0.08, 0.10, 0.12, etc., through 12 tubes. The titration was incubated one hour at 37° C and two full units used for the test measured as follows: if the tube just cleared, in which two units of undiluted complement would be reckoned as 0.016 c c per tube, then 0.017 c c one point farther was used per tube in the series.

Ice-box fixation was four hours.

Two units of amboceptor were used according to titration. A uniform titre was employed and each new amboceptor was titrated against the previous one.

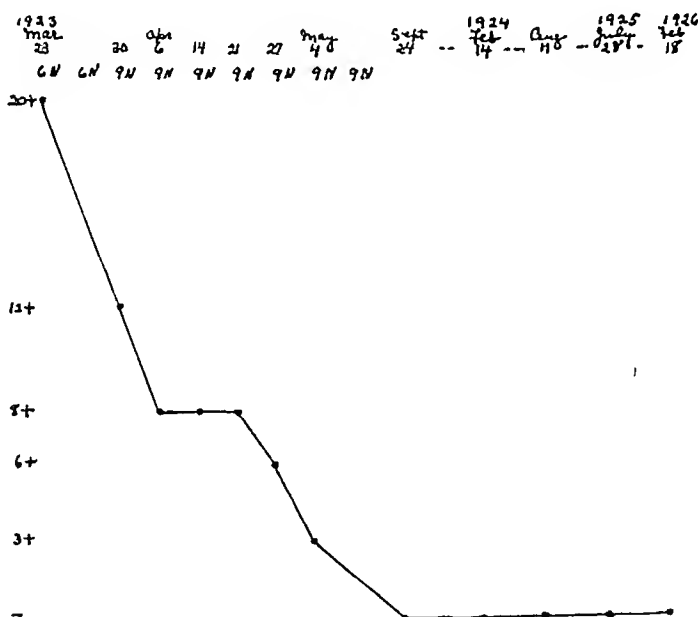


Chart 4—Patient C had the rash of the secondary stage of syphilis at the time the first Wassermann was made March 23 1923. The reaction was strongly positive titrating 20-plus. He was given neosalvarsamine 0.6 gm for the first two injections then 0.9 gm at weekly intervals until May 11. He used mercuric ointment inunctions about every other day from the first week until May. He also took the protiodide of mercury by mouth keeping himself to the point of saturation. After May he took no more neosalvarsan and also stopped the inunctions. He continued taking mercury by mouth as much as he could tolerate until September. On Sept. 24 the Wassermann was negative. He continued the use of mercury until February 1924. Since then he has had no treatment of any kind and his Wassermann has remained negative.

The curve of titration is shown in Chart 4.

Five per cent washed sheep's cells were used in the first series and 2½ per cent in the second series. A suitable packing of corpuscles was obtained with our centrifuge by twenty minutes on speed one. The cells were sensitized. The corpuscle suspension was strained through gauze before adding to the amboceptor.

Incubation was one-half hour in the water-bath at 37° C.

There were the usual positive and negative controls, using a moderately positive serum for the positive control.

Those tests which gave a one-plus or a very weak two-plus with the cholesterolized antigen only, were not included in the percentage of positives. Tables I and II show the percentages.

TABLE I

	TOTAL POSITIVES	0 1% CHOLESTEROLIZED	KOLMER	PLAIN ALCOHOLIC	ACETONE INSOLUBLE
1st series	1517	1512	1471	1283	1042
5000 tests	30 34%	30 24%	29 42%	25 66%	20 84%
2nd series	1386	1385	1080	1190	826
5000 tests	27 72%	27 7%	21 6%	23 8%	16 52%

TABLE II

	0 1% CHOLESTEROLIZED	KOLMER	PLAIN ALCOHOLIC	ACETONE INSOLUBLE
1st series	1512	1471	1283	1042
1517 positives	99 67%	96 96%	84 57%	68 68%
2nd series	1385	1080	1190	826
1386 positives	99 92%	77 92%	88 85%	59 59%

The most uniform results were given by the 0.1 per cent cholesterolized antigen. Dr. Kolmer's own antigen, obtained from him, compared favorably with the 0.1 per cent cholesterolized product, but Kolmer's antigen prepared after his technique did not give as good results.

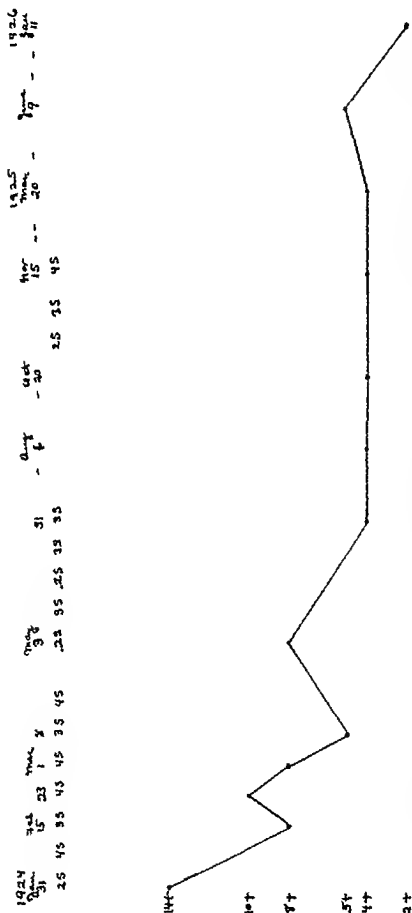
There was considerable variation between the antigenic value of Dr. Kolmer's own antigen and those made after his technique without the use of the Soxhlet or shaking machine. The omission of the mechanical shaker was the most obvious cause of variation, and differences in the value of the powdered muscle may also have been a factor. A third source of variability may have been the acetone insoluble lipid portion of the Kolmer preparation, since there is often considerable differences in the antigenic value of the acetone insoluble antigens.

It has been brought to our attention that other workers confining themselves to the Kolmer test may be using an antigen which is not of uniform sensitivity in comparison with Dr. Kolmer's own preparation. For instance, several times we have had an opportunity to test a serum upon which some other serologist using the Kolmer technique alone returned a frank negative report. In each case, our results were four-plus positive with three antigens, including a Kolmer preparation of the second series. The positive reaction agreed with the clinical findings. In one case, a third laboratory using a group antigen system returned also a four-plus positive.

If a worker confines himself to one antigen he may be using one with less antigenic value for some time without really knowing it unless he has some means of checking his results. The titration figure is not always a sure indication of the sensitivity of an antigen.

In one of Dr. Kolmer's antigens prepared in his laboratory, ten units were represented by a dilution of 1 to 220. In another one ten units were represented by a dilution of 1 to 320. Both were sensitive. A Kolmer preparation of the second series titrated under the same conditions with 0.1 per

cent cholesterolized antigen, using the same positive sera gave a four plus reaction in a dilution of 1 to 2500 while the cholesterolized gave a four plus in a dilution only as high 1 1000 yet the latter antigen was far more sensitive



PART III

There are three quantitative methods for titrating positivity of a Wassermann reaction. First, by using graduated amounts of serum and a fixed amount of complement and antigen. This is the usual procedure. Second, by using graduated amounts of antigen and a fixed quantity of serum and complement. Third by using graduated amounts of complement and fixed portions of serum and antigen. This last method is the one we followed.

Browning and McKenzie⁶ are particularly associated with the use of graduated portions of complement with fixed quantities of serum and antigen. In their routine tests they used three amounts of complement 2, 4 and 6 units.

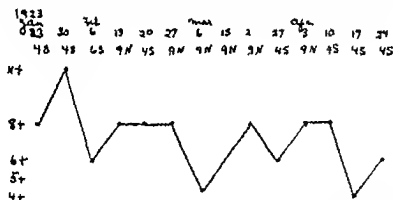


Chart 9—Patient H was an old case of syphilis that had received other courses of treatment. He was sent to the laboratory for a course of arsphenamine and neosalvarsan injections. He had fourteen injections at weekly intervals. The doses were as follows: Jan 3 and 30 0.4 gm German salvarsan; Feb 6 0.6 gm; Feb 13, 0.9 gm German neosalvarsan; Feb 27 0.4 gm salvarsan; Feb 27 March 6 1.3 and 0.9 gm neosalvarsan; March 27 0.4 gm salvarsan; April 3 0.9 gm neosalvarsan; April 10 1.7 and 4.0 gm salvarsan. The blood for the Wassermann was withdrawn before each injection. The curve of titration is seen in Chart 9.

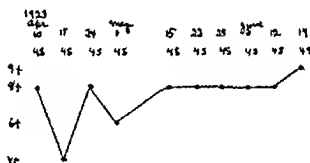


Chart 10—Patient I was an old case of syphilis that had received other courses of treatment. He was sent to the laboratory April 10, 1923 for a course of ten injections of salvarsan. The Wassermann was strongly positive titrating 8 plus 0.4 gm German arsphenamine was given at weekly intervals with one exception. The treatment May 8 was omitted. The blood for the Wassermann was withdrawn before each injection.

The curve of titration is seen in Chart 10.

Dr Kolmer⁸ in discussing Browning and McKenzie's method states "it was very satisfactory and with a few modifications not involving the essential principles, yielded good results in his hands." He also states "that the main advantages are the strictly quantitative character of the test and the possibility of examining anticomplementary sera; the main disadvantages are the expense involving the use of large amounts of complement serum where many sera are to be tested and difficulties in reading permitting variation due to personal equation." We would mention one other objection in regard to using increases

ing doses of complement, the fact that one is also using considerable amounts of variable biologic material

This method was of interest to us, however, because it could be made a simple procedure and the way the complement was used, the reckoning of the degree of positivity appeared to us logical and easy

We applied the method in the following manner

In each tube was placed 0.1 cc of serum and the solution containing ten units of antigen. To each tube was added respectively, two, four, six, eight, ten and twelve units of complement, six tubes being often sufficient. Two units of complement were taken as the standard. When these tubes came to the reading, it just two units of complement were completely bound by the syphilitic reagent and antigen complex that represented a four-plus positive. If four units were completely bound that represented an additional four-plus, making a result of eight-plus. If for instance, ten units were bound the results would be recorded as twenty-plus. If only fifty per cent of tube 5 was bound the positivity was reckoned as eighteen-plus instead of twenty-plus.

Table III shows the method more graphically

TABLE III

	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5	TUBE 6
Serum	0.1 cc	0.1 cc	0.1 cc	0.1 cc	0.1 cc	0.1 cc
Antigen	10 units	10 units	10 units	10 units	10 units	10 units
Complement	2 units	4 units	6 units	8 units	10 units	12 units
Amount of positivity	4	8	12	16	20	24

It is necessary for the serologist to use a uniform method of titrating complement, a uniform antigen and a uniform length of fixation period for all titrations of one given case. In our series the plain alcoholic antigen was employed because we wished to use an antigen generally recognized at the time the work was begun, as giving good average results.

The general line of procedure and titration of complement in the accompanying cases was the same as outlined in Part II. The plain alcoholic antigen, as described in Part II, and four-hour ice-box fixation was employed. The complement dose was added with a 0.2 cc pipette graduated in one thousandths and salt was added to make 0.5 cc which was the complement volume per tube.

Some of the results of this work are presented in the accompanying charts.

The details of treatment are given in the cases only during the period the titration curve appeared of interest. The first six charts illustrate the titration curve in cases of early syphilis.

If the blood was procured before the first treatment and also before the second treatment, a rise in positivity was often apparent as shown in Charts 1 and 2. There was usually an abrupt descent of the curve after two or three salvarsan injections. Then there was a period of two, three, four or more treatments when the curve showed little change and this period was followed usually by a gradual descent to a negative reading.

The results in patients with late or latent syphilis, either untreated or

previously treated, were not so satisfactory. Charts 7, 8, 9 and 10 represent this type of case.

Chart 7 of patient F was a case who had never received any previous treatment. The result indicated a general downward trend and was better than in the other three cases. They all showed a fluctuating response in the early stage of the injections and then a continued fluctuating result or a tendency to remain without much change. Titrations 8, 9 and 10 gave no indication of becoming negative. The curve in Chart 10 at the end of a course of salvarsan was higher than at the beginning.

A number of reasons may be suggested for the fluctuating curve in the old, persistently positive case. First, variability in dosage and changes from one drug to another. Second, the omission of a treatment as between October 10 and 24 in Chart 8 and between May 1 and 15 in Chart 10. In both there was a rise in the curve. Again the syphilitic reaction may vary in its response to the treatment, yielding temporarily, then undergo a readjustment, following somewhat the swing of a pendulum. It also may be particularly sensitive to differences in complement. Finally, the hydrogen ion content of the salt may be a factor in variation.

When there was a marked fluctuation in the titration curve for no apparent reason, the titration was repeated to verify the result.

SUMMARY

PART I

Some routine features in connection with Wassermann technique can often be reiterated to advantage.

PART II

1. Dr. Kolmer's antigen as prepared in his laboratory compared favorably with the 0.1 per cent cholesterolized antigen with the four hour ice box fixation and was considerably more sensitive than the plain alcoholic and acetone insoluble antigens.

2. The three antigens prepared after the Kolmer technique without the use of the mechanical shaker showed much less antigenic value than the Kolmer antigens prepared in Dr. Kolmer's laboratory.

3. The most obvious cause of variation was the omission of the mechanical shaker. Other factors may have been differences in value of the powdered muscle and variability in the acetone insoluble lipid portion of the preparation, since acetone insoluble antigens are known to vary markedly in sensitivity.

4. Inasmuch as antigens do vary as prepared in different laboratories and since the titration figure does not always give complete information as to the sensitivity of an antigen, the use of group antigens is an aid in checking the antigenic value of a given antigen and in producing reliable Wassermann results.

PART III

1. The method of using fixed amounts of serum and antigen respectively and of uniformly increasing the doses of complement two units through a series of tubes offers a simple way of titrating the positivity of a serum.

2 The reckoning of positivity is made an easy procedure by adding four plus to the positivity of a serum for every additional two units of complement which are bound by the serum and antigen complex

3 The titration curves compiled through the treatment of a series of cases showed more uniform and satisfactory results in the case of early syphilis than in the old case either previously untreated or treated

NOTE We desire to express our appreciation to Dr Josiah J Moore, through whose courtesy and generous assistance these cases are presented

REFERENCES

- ¹Kahn, R L A Simple Method for the Removal of Natural Amboceptor from Human Sera, *JOUR LAB AND CLIN MED*, 1920, vi, 218
- ²Kolmer, John A A Superior Antigen for Complement Fixation Tests in Syphilis, *Am Jour Syph*, 1922, vi, 74-82
- ³Neymanu, C A and Gager, L I A New Method for Making Wassermann Antigens from Normal Heart Tissue, *Jour Immun*, 1917, ii, 573-583
- ⁴Kolmer, John A A New Complement Fixation Test for Syphilis, Based upon the Results of Studies in the Standardization of Technique, *Am Jour Syph*, 1922, vi, 82-111
- ⁵Browning, Carl H, and McKenzie, Ivy The Biological Syphilis Reaction, Its Significance and Method of Application, *London Lancet*, 1909, i, 1521-1523
- ⁶Browning, C H, and McKenzie, I Recent Methods in Diagnosis and Treatment of Syphilis, Lea and Febiger, 1913, p 13
- ⁷Browning, C H, and Witson Venereal Diseases, 1919, pp 103, 302
- ⁸Kolmer, John A A Study of Methods for Conducting Quantitative Complement Fixation Tests and of Reading Scales for Recording Reactions, *Am Jour Syph*, 1922, vi, 65

METASTASIS TO THYROID GLAND FROM ENDOTHELIAL MYELOMA OF BONE, RAPID REGRESSION RESULTING FROM ROENTGEN-RAY TREATMENT

BY LLOYD F CRAVER, M D, NEW YORK CITY*

THE case reported below has several unusual features. An interesting problem in diagnosis was presented, because the patient had an arm amputated for supposed periosteal sarcoma in one hospital, and an operation done on the thyroid in another hospital for supposed carcinoma of the thyroid, and came to Memorial Hospital with a large mediastinal tumor said to be an extension of the carcinoma of the thyroid. It appeared at once that in all probability one or both of the former diagnoses were wrong, as it was much more likely that both tumors were the same. By taking pains to secure the sections from both hospitals, it was possible to prove this assumption correct. The comparative rarity, not only of endothelial myeloma of bone, but particularly of metastases of any tumor to the thyroid gland, adds further to the unusual features of this case. Finally, the rapid regression of the mediastinal mass under roentgen-ray treatment affords an illustration of the remarkable radiosensitiveness of endothelial myeloma.

CASE REPORT

The patient was a man of thirty, a native of Holland, and was referred to Memorial Hospital on August 31, 1926, from another institution where, one month before, he had been operated on for a supposed carcinoma of the thyroid.

*Attending Physician Memorial Hospital
Received for publication January 11 1927

The history of the tumor in the thyroid was as follows. About three months before the operation he had found a small lump in the thyroid region. It had not been painful and at times seemed to subside, until about three weeks before the operation when the swelling had increased and from then until the time of the operation it had persisted without remission. It had become about the size of a small orange and involved the right lobe of the thyroid being attached to the trachea which was displaced medially to the left. The mass was hard, and the patient had great difficulty in swallowing.

At operation the tumor was found to lie within the thyroid substance and was attached to the trachea and the cervical vertebrae. A mass of tumor about 8x5x4 cm was removed but an attempt to remove masses extending within the mediastinum was unsuccessful. The

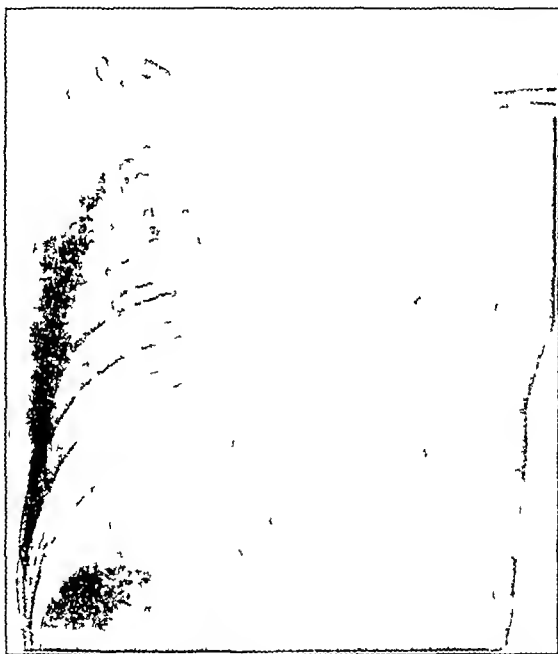


Fig 1—Roentgen film of chest showing large mass in mediastinum continuous with mass felt beneath scar in neck. Compare with Fig

section showed mostly firm dark red tissue except at one end where there was pale firm tissue. The microscopic examination was reported as showing carcinoma of the thyroid (probably because of the presence at one point of thyroid tissue with cystic acini containing colloid).

Upon the arrival of the patient at Memorial Hospital one month after the operation it was evident that although he was able to be up and about he was acutely ill. He suffered from marked dyspnea with orthopnea and a severe cough. Under the scar of the recent operation there was still present in the thyroid region a considerable thickening and physical signs confirmed by the roentgen ray (Fig 1) indicated the presence of a large mass in the anterior mediastinum extending further to the right and continuous with the mass beneath the scar.

Treatment was begun immediately, without waiting to clear up the discrepancies in the diagnosis. A water cooled high voltage Coolidge tube was used, with the following factors: 200 kilovolts, 20 milliamperes, 50 cm target skin distance, and filtration of 0.5 mm copper and 0.5 mm aluminum. Because of the pressure symptoms it was thought unsafe to give more than five minutes, i.e., about $\frac{1}{3}$ an erythema dose, for the first exposure. This was given directly over the thyroid and mediastinal mass anteriorly.

The remarkable effect of this treatment was strikingly shown by the prompt lessening of pressure symptoms. Because of orthopnea the first treatment had to be given to the patient as he sat in a chair. Three days later he could lie down with perfect comfort for the second treatment. A third exposure of five minutes completed the first series of treatments. The



Fig 2—Roentgen film of chest taken Oct 13 1926 about five weeks after first series of high voltage roentgen treatments. Compare with Fig 1.

rapid regression of the tumor continued, as shown by films made ten days and about five weeks following the first series of treatments. (See Figs 1 and 2). Sixteen days after the third treatment an additional series of 4 exposures was given, from five to eight minutes each, to the sides of the neck and posteriorly to the mediastinum. Simultaneously with the rapid regression of the tumor there was marked relief from symptoms, in fact a complete disappearance of dyspnea and cough, and there was pronounced gain in weight and strength. The patient was soon able to resume his duties as a janitor, and has remained apparently well for over three months. Certainly even this brief palliation has been worth while.

In the meantime, further investigation of the nature of the disease was being carried out. The right arm had been amputated at a third institution 17 months before (in March,

1925) A report from that hospital revealed that nearly four years ago the right humerus was explored under a preoperative diagnosis of periostitis the curettings having been reported as infected and necrotic pieces of bone. Again in March 1925 the humerus had been explored, and as the pathologic report was periosteal sarcoma the arm was amputated.

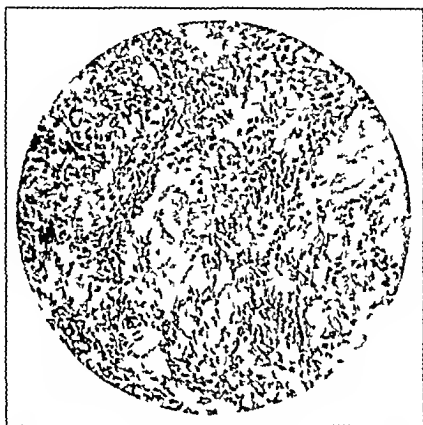


Fig 3—Microphotograph of section from the tumor removed from the thyroid gland. High power.

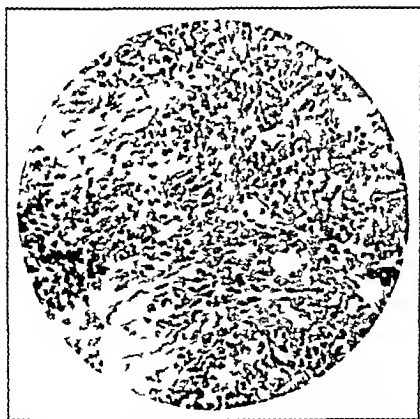


Fig 4—Microphotograph of section from tumor of humerus. High power.

When the patient came to Memorial Hospital it was felt that in all probability the thyroid tumor and the tumor of the humerus were of the same origin but it was hard to reconcile a diagnosis of periosteal sarcoma with that of carcinoma of the thyroid. Disregarding entirely the previous reports and considering only that there had been a malignant tumor

of the humerus and of the thyroid, probably one and the same in nature, the question arose, was this a primary carcinoma of the thyroid which had first given symptoms by a metastasis to the humerus, or was it a primary sarcoma of the humerus which had metastasized to the thyroid? The absence of evidence of disease elsewhere made it seem unlikely that the third possibility need be considered, namely, a tumor arising neither in humerus nor thyroid, and metastasizing to both.

After some delay, the sections were secured from both hospitals and submitted to Dr James Ewing, whose report follows: "The thyroid tumor is an extremely cellular, malignant anaplastic growth, identical in type with that of the humerus. It is composed of sheets of medium sized round or polyhedral cells. These cells are composed mostly of nucleus. They surround small capillaries, simulating a papillary adenocarcinoma. No definite epithelial qualities. General impression is that of endothelial myeloma." (Figs 3 and 4)

This case is not reported as a cure, as it is realized that the duration of the favorable result (a little over three months) is far too short to justify any such claim. Rapid recurrence is to be expected in this type of tumor, and undoubtedly further treatment will be required. The intent of this report is to put on record the combination of unusual features indicated at the beginning of this paper.

Addendum—April 18, 1927. Further treatment to the mediastinum was given during February, 1927, because there was a slight increase in the width of the mediastinal shadow and some cough. More recently a metastasis has appeared at the base of the left lung.

SOME NOTES ON GLYCOL, GLYCOL-CHLORETONE ANESTHESIA*

BY H. B. HAAG AND W. R. BOND, RICHMOND, VA.

THE last few years have witnessed an increased zeal in the quest of a suitable substitute for ethyl alcohol. Among the many substances which have been advanced for this purpose is ethylene glycol, a dihydric alcohol discovered by Wurtz in 1856. The possibilities of glycol were never strongly stressed until Bachem¹ suggested several uses, especially remarking on its adaptability as a substitute for glycerin. He noted that it could be used in the place of glycerin in preparing suppositories, ointments, and other pharmaceuticals. Administered orally to dogs in a dose of 5 cc per kg body weight, no untoward effects were observed. He also stated that it had a slight laxative action, similar to that produced by glycerin. Franck later advised its use as a source of food and as a substitute for ethyl alcohol. Wolff,² in 1920, again called attention to the possibilities of glycol in the preparation of medicinals. Cueme³ mentioned its prospects as a solvent, preservative and organic base. Fuller⁴ declared that as a preservative against molds, yeasts and bacteria, it approaches ethyl alcohol, and is superior to glycerin. The substance has of late gained considerable favor as an antifreeze compound, being superior to glycerin because of its lower molecular weight.

Ethylene glycol (glycol, ethanedol) has a formula $C_2H_4(OH)_2$. Physically it is a colorless, odorless, syrupy, hygroscopic liquid having a sweet taste. It is soluble in water and ethyl alcohol, but only slightly so in ether. The compound has a boiling point of $198^\circ C$ and a freezing point of $-13^\circ C$. It is somewhat more volatile and less viscous than glycerin. The specific gravity is 1.115.

*From the Department of Pharmacology, Medical College of Virginia, Richmond, Va.
Received for publication January 25, 1927.

Used in our laboratory as a substitute for ethyl alcohol in the preparation of tr digitalis, glycol, as judged by the results of several cat assays, proved somewhat disappointing. Glycol apparently does not have the same solvent action on the active principles of digitalis as does ethyl alcohol. The substitution of glycol for alcohol in spt nitrous ether and in tr iodine was found to be impossible. These were the only official preparations in which substitution was attempted. It was found however that glycol will not mix with balsam of Peru, or camphor. It will mix with menthol, ichthyol, the waters, phenol and chloretone. Aspirin is fairly soluble.

Experiments conducted in our laboratory have led us to the belief that glycol has an extremely low toxicity. Intraperitoneal administration to dogs in amounts of 5 cc per kg body weight apparently showed no injurious effects. Intravenous injections of 15 cc per kg body weight were tolerated with little or no discomfort. Tracings of blood pressure indicate no appreciable change in pressure following the slow intravenous injection of 15 cc per kg body weight.

For the past two years we have used a glycol solution of chloretone as an anesthetic for dogs and cats in conducting such laboratory experiments in physiology and pharmacology as the use of chloretone will permit. It has been our experience that 0.5 cc of a 40 per cent solution in glycol per kg body weight injected intraperitoneally suffices to maintain the animals in a satisfactory state of anesthesia. From observations of several hundred animals, this mixture seems to possess all the advantages and none of the disadvantages of the older 10 per cent chloretone olive oil solutions. Because of the greater concentration possible with glycol the volume injected is decreased making administration quicker and easier. The higher mobility of the glycol mixture allows the use of smaller needles. This and the fact that the solution is but slightly irritating makes the preliminary injection of morphine unnecessary. We have been led to believe that the glycol mixture produces its effects more quickly than the chloretone olive oil solutions. While most of the animals injected apparently suffered no discomfort occasionally one was encountered which reacted somewhat unfavorably, pain being marked. In these cases we believe that the peritoneum had been mechanically traumatized by the process of injection, and that this pain was not the result of irritation due to the glycol chloretone mixture. The amount of olive oil or cottonseed oil employed in 10 per cent solutions often proved annoying in experiments on the abdominal viscera, this difficulty is obviated by use of the glycol mixture. Advantage here is due to the small amount necessary, the fact that glycol is fairly rapidly absorbed and to its miscibility with the peritoneal fluid.

Should there be an adequate demand for glycol it could probably be dispensed at a price lower than either glycerol or alcohol. This alone should stimulate research in an endeavor to determine its further possibilities.

REFERENCES

- ¹Bachem, C. München med. Wchnschr. 1916 lxxv, 1475
- ²Franck, H. H. München med. Wchnschr. 1918 lxxv, 1216
- ³Wolff, H. Seifenfabr. 1920, xl, 245-6
- ⁴Curme, G. O., Jr., and Young, C. C. Chem. and Met. Eng., 1923 xxviii, 169-70
- ⁵Fuller, H. C. Ind. and Eng. Chem. 1924, xvi, 24

NOTES ON BASAL METABOLISM, IX * SIMPLIFIED CALCULATION FOR GASOMETER GAS ANALYSIS METHOD†

BY WILLIAM H. STONER, A.M., M.D., PHILADELPHIA, PA.

FORMULAS of maximum simplicity have been developed for calculating, by means of a slide rule, respiratory quotient and basal metabolic rate from the observed data of the open circuit and gas analysis method for measuring respiratory exchange.

The usual step by step calculation is the logarithmic one of Boothby and Sandiford¹ or one of its many modifications². These methods appear to be duly laborious and time consuming. Logarithms of the various observed values and constants are added or subtracted in succession without an attempt either to collect added and subtracted logarithms or to combine constants common to every determination. In this way the respiratory quotient and basal metabolic rate are derived by a multitude of operations starting with the observed data and progressing logically to the desired result. Such a procedure, of course, makes the rationale of the calculation perfectly obvious and has been stated³ to have the advantage of giving a complete record in each determination of all the various partial values, as ventilation per minute, calories per hour or day and calories per hour per square meter body surface. Since it is not apparent what use could be made of these partial values and since, if they were wanted, they could be obtained readily by a single slide rule setting upon the final result, it seems that this slight advantage scarcely justifies the added labor of calculating and recording. It would appear to be more expeditious to derive simple formulas based upon the rationale of the calculation and to substitute simply the observed values of a determination in these formulas for respiratory quotient and basal metabolic rate.

Several items are important in deriving simple formulas for such calculations. First, all values common to every determination should be combined into one constant.

Second, as many of the factors as possible should be made constant and

*From the Biochemistry Laboratory and the Department of Metabolic Diseases of the Graduate School of Medicine of the University of Pennsylvania.

Received for publication January 24, 1927.

†Preceding Notes of this series appeared as follows:

- I Modified Clinical Method of Determination Boston Med and Surg Jour 1923 clxxxix 193
- II A Simplified Data Card for Clinical Determination Boston Med and Surg Jour 1923 clxxxix 195
- III Errors of Clinical Determination Boston Med and Surg Jour 1923 clxxxix 232
- IV Selection of Normal Standards Boston Med and Surg Jour 1923 clxxxix 236
- V Tables of Values for Dreyer's Formulas Boston Med and Surg Jour 1923 clxxxix 239
- VI Complementary Tables of Values of Dreyer's Formulas Boston Med and Surg Jour 1924 cxci 1026
- VII Actual Versus Theoretic Weight in Dreyer's Formulas Boston Med and Surg Jour 1924 cxci 1030
- VIII Tables of Values of the Dubois Surface Area Formula JOUR. LAB AND CLIN MED 1926 xl 355

included in the one constant of the final formula. Thus, time (duration of the test), which, in the usual logarithmic forms for this calculation is made a variable, may be made a constant as ten, twelve or fifteen minutes, without sacrificing anything of accuracy or convenience in manipulation. Again, the gasometer factor may be included in the general constant substituting simple linear measure of gasometer bell rise for volume in the final formula for basal metabolic rate. In this way these values time and gasometer factor become absorbed into the formula constant and two less operations are required in every determination.

Third, the necessity for referring to tables should be minimized. For example, the one half of one per cent of accuracy gained in correcting a barometer reading for temperature scarcely justifies the expenditure of time consumed in consulting a table to determine whether 2 or 3 millimeters should be used in the correction of the observed barometric pressure. It would seem sufficiently accurate either to ignore this correction or to correct routinely by subtracting either 2 or 3 millimeters without reference to a table. That errors of many times this magnitude are inherent and inevitable in the determination of basal metabolic rate was shown in the third note of this series. Again the use of logarithms for the solution of the two digit respiratory quotient or of the three digit basal metabolic rate seems unnecessarily meticulous when identical results are obtained in considerably less time by means of a slide rule or of one of the ordinary commercial adding machines. Furthermore reference to tabular values of corrections of volumes for temperature and pressure is unnecessary. The values of normal temperature 273 absolute and normal pressure 760 millimeters or 29.92 inches may be included in the general constant of the final formula and the observed temperature and barometric pressure corrected for tension of aqueous vapor (and if desired for temperature) are carried as literal variable values in the formula. Another unnecessary table generally used in the calculation of respiratory quotient is one giving volume of oxygen of inspired air corresponding to the nitrogen per cent in expired air. This table is ignored in the calculation given here. The only fact required is that the volumes of inspired and expired air are inversely proportional to their nitrogen percentages.

Fourth the form of the final formula should be such that a minimum of kinds of operations necessary for its solution is required. For solution by means of the slide rule the simplest formula is one which has no processes of addition or subtraction which cannot be performed by inspection and requires only multiplication and division. This statement also applies to solution of formulas by logarithms. The formulas given here may be solved logarithmically if desired.

Fifth, it is well known that for the solution by slide rule of a formula having several simple numerical factors in the numerator and in the denominator several settings of the rule are eliminated by alternate multiplication and division. For this reason it is advantageous to have approximately the same number of factors in the numerator as in the denominator. It is for this reason that the general constant of the final formula for basal metabolic rate given here is included in the denominator rather than in the numerator.

DEVELOPMENT OF FORMULAS

For purposes of explanation and brevity the following letters are assigned the various values specified

C = percentage by volume of CO_2 in expired air

O = " " " " O_2 " " "

N = " " " " N_2 " " "

v = liters of air at observed temperature and pressure expired in 10 minutes

t = observed absolute temperature of expired air at time of measurement

p = observed barometric pressure minus tension of aqueous vapor at t (The scrupulous correction for barometric temperature may also be made)

V_t = liters, at normal temperature and pressure, of air inspired in 10 minutes

V_e = liters, at normal temperature and pressure of air expired in 10 minutes

c = caloric value of 1 liter of O_2 at normal temperature and pressure and observed respiratory quotient (An error of approximately 1 per cent may be avoided by correcting this value on the basis of the assumption that approximately 15 per cent of the calories are derived from protein⁷⁻⁹)

f = $0.2648 N - O$

H = expected calories per day according to formulas of Harris and Benedict^{1 3 4 10 13} or Dreyer^{3 4 14-15}

A = expected calories per hour per square meter according to table of Aub and DuBois^{1 3 4 11-13 16 17}

S = surface area in square meters according to formula of DuBois and DuBois^{1 3 4 11-13 18 19}

A RESPIRATORY QUOTIENT

There is a rather widespread lack of recognition of the fundamental fact that respiratory quotient is a ratio and is entirely unrelated either to the volume of expired or inspired air or to the time duration of the test. So that to determine respiratory quotient these two measurements are unnecessary so long as the duration of the test is long enough to yield a volume of expired air sufficiently large to make the sample representative. The recognition of this fact is of particular value in the more recent studies²⁰ of respiratory quotient curves before and after dextrose administration in the differential diagnosis between renal glycosuria and mild diabetes mellitus. In these studies, if the heat production is not required, volume of expired air and duration of test need not be measured.

Definition—Respiratory quotient is the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed in a given time. Outside air which is inhaled in the test has the universal composition of 20.93 per cent by volume of oxygen, 0.04 per cent of carbon dioxide and 79.03 per cent of nitrogen. The volume (not the per cent) of nitrogen inhaled is equal to the volume of nitrogen exhaled.

Volume of carbon dioxide produced = $0.01 CV_e - 0.0004 V_t$
and " " oxygen consumed = $0.2093 V_t - 0.01 OV_e$

By definition, respiratory quotient = $\frac{0.01 CV_e - 0.0004 V_t}{0.2093 V_t - 0.01 OV_e}$

$$\text{or } R Q = \frac{OV_e - 0.04 V_t}{20.93 V_t - OV_e} \quad (1)$$

Since the volume of nitrogen in inspired air is equal to volume of nitrogen in expired air,

$$0.7903 V_t = 0.01 NV_e \quad \text{or } V_t = \frac{NV_e}{79.03}$$

Substituting this value of V_t in (1),

$$R Q = \frac{C V_e - \frac{0.04 N V_e}{79.03}}{\frac{20.93 N V_e}{79.03} - C V_e} = \frac{79.03 C - 0.04 N}{20.93 N - 79.03 O}$$

$$\text{or } R Q = \frac{C - 0.0005 N}{0.2648 N - O}$$

Since the value $0.0005 N$ is always 0.04 ,

$$R Q = \frac{C - 0.04}{0.2648 N - O}$$

The simplest and quickest method of solving this formula has proved to be by means of a commercial calculating machine which is also used in calculating the gas analytic results. The burette readings of (1) sample, (2) volume after absorption of CO_2 and (3) after absorption of O are recorded on paper and corrected. Their subtraction and the division of the remainders by the volume of the sample are performed on the calculating machine, and, immediately, from the resulting values for C , O and N the respiratory quotient formula is calculated by the same machine.

B. BASAL METABOLIC RATE

Liters of oxygen at normal temperature and pressure consumed in 10 minutes = $0.2093 V_t - 0.01 O V_e$

Substituting value of $V_t = \frac{N V_e}{79.03}$

$$\frac{0.2093 N V_e}{79.03} - 0.01 O V_e = 0.01 V_e (0.2648 N - O)$$

Since $V_e = \frac{v p 273}{760 t}$ (correction for temperature and pressure)

Liters of oxygen consumed in 10 minutes reduced to normal temperature and pressure = $\frac{0.01 v p 273 (0.2648 N - O)}{760 t}$

Multiplying this value by 144 gives the oxygen consumption per day, multiplying by c gives the calories per day, multiplying by 100 dividing by H and subtracting 100 from the whole value gives the percentile variation of actual number of daily calories from the expected number which is basal metabolic rate and representing the factor $(0.2648 N - O)$ which is the denominator in the $R Q$ formula by f

$$B M R = \frac{0.01 v p 273 (0.2648 N - O) 144 c 100}{760 t H} - 100$$

$$\text{Or } B M R = \frac{v p f c}{0.0193 t H} - 100$$

The above formula applies to the Harris and Benedict standards. If Dreyer standards are used, D is simply substituted for H . If the Aub and DuBois standards are used the formula becomes

$$B M R = \frac{v p f c}{0.463 t A B} - 100$$

These formulas are probably solved with greatest facility by means of a slide rule but if several are to be calculated at the same time much speed may be made by use of the calculating machine to add and subtract the four place

logarithms of the values as found without writing them. As is usual in such routine calculations the characteristics of the logarithms and the decimal point in the slide rule calculation may be ignored.

SUMMARY

Simplified formulas are developed for calculating respiratory quotient and basal metabolic rate from gas analytic and gasometric data.

REFERENCES

- ¹Boothby, Walter M, and Sandiford, Irene. Laboratory Manual of the Technique of Basal Metabolic Rate Determinations, Philadelphia, 1920.
- ²Bailey, Cameron V. JOUR LAB AND CLIN MED, 1921, vi, 657.
- ³Hawk, Philip B, and Bergeim, Olaf. Practical Physiologic Chemistry, Philadelphia, 1926.
- ⁴Sanborn, Frank B. Basal Metabolism, Its Determination and Application, Boston, 1922, p 260.
- ⁵Gradwohl, R B H, and Blaivas, A J. Blood and Urine Chemistry, St Louis, 1920, 378.
- ⁶Boothby, Walter M. Personal communication.
- ⁷DuBois, Eugene F. Basal Metabolism in Health and Disease, Philadelphia, 1924, 38, 70, 72.
- ⁸Michaëlis, A M. Jour Biol Chem, 1924, lx, 51.
- ⁹Magnus Levy. Van Noorden's Handbuch, 1901, 1, 192.
- ¹⁰Harris, J Arthur, and Benedict, Francis G. A Biometric Study of Basal Metabolism in Man, Carnegie Institution of Washington, 1919, Pub No 279.
- ¹¹Carpenter, Thorne M. Tables, Factors, and Formulas for Computing Respiratory Exchange and Biological Transformations of Energy, Carnegie Institution of Washington, 1921 and 1924, Pub No 303, and 303A.
- ¹²Joslin, Elliott P. Treatment of Diabetes Mellitus, Philadelphia, 1923.
- ¹³Benedict, Francis G. Abderhalden's Handbuch, Abt iv, Teil 10.
- ¹⁴Dreyer, Georges. Lancet, 1920, cxc, 289.
- ¹⁵Stoner, William H. Boston Med and Surg Jour, 1923, clxxxix, 239, 1924, cxci, 1026, 1030.
- ¹⁶Aub, J C, and DuBois, E F. Arch Int Med, 1917, xiv, 823.
- ¹⁷Stoner, William H. Boston Med and Surg Jour, 1923, clxxxix, 236.
- ¹⁸DuBois, Delafield, and DuBois, Eugene F. Arch Int Med, 1916, xvi, 863.
- ¹⁹Stoner, William H. JOUR LAB AND CLIN MED, 1926, ix, 355.
- ²⁰Petty, Orlando H, and Stoner, William H. Am Jour Med Sci, 1926, clxvi, 842.

LABORATORY METHODS

A STANDARD METHOD OF RECORDING THE HEMOGLOBIN CONTENT OF BLOOD*

By C A ELVEHJEM AND J WADDELL PH D MADISON WIS †

IN MUCH of the experimental work that is being done in various laboratories on the factors effecting changes in the blood stream many hemoglobin determinations are made. These determinations are made on the blood of different species of animals and several different hemoglobinometers are made use of. It has occurred to us that data presented from various sources would be more uniform and carry more real information if a standard method of recording hemoglobin values were followed.

The earlier hemoglobinometers were elaborated chiefly for use in clinical laboratories where examinations on human blood were made. It is understandable, therefore, that the custom arose of expressing results in terms of a so called "normal" for human blood. Many different hemoglobinometers however, have now come into use and with them almost as many standards. These standards vary depending upon the amount of hemoglobin chosen to represent the normal amount in human blood. Thus the reading of 100 per cent from two different instruments does not represent the same amount of hemoglobin unless they happen to make use of the same standard for human blood which is unusual. For example 100 per cent of hemoglobin would equal the following grams of hemoglobin per 100 cc of blood in the different instruments¹

Dare	13 77
Haldane	13 80
Oliver	15 00
Von Fleischl Miescher	15 80
Tallqvist	15 80
Sahl	17 20
Newcomer	16 92

The confusion is further increased when one attempts to express the amount of hemoglobin found in the blood of different species in terms of percentage of a normal for human blood. Thus the hemoglobin contained in the blood

Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

¹From the Department of Agricultural Chemistry, University of Wisconsin.

Received for publication January 19 1917

of a normal rabbit or a normal chicken might read well below 100 per cent of the standard while that of a normal rat might read well over 100 per cent. This situation has been brought home to us since we have had occasion to make numerous determinations on different species in this laboratory.

From a chemical standpoint it would seem to be more logical to express the amount of hemoglobin found as grams per 100 c.c. of blood. This means of expression is that commonly used for many other blood constituents and has been proposed previously as a method that should be followed in recording hemoglobin values. Williamson² as early as 1916 made the suggestion and recently many clinicians³ have made a strong plea for this standard means of expressing the amount of hemoglobin.

The Von Fleischl-Miescher hemoglobinometer gives a reading of grams per 100 c.c. directly and the Newcomer instrument gives a similar reading when a special conversion table is used, but in many of the other instruments only the reading of the per cent of normal is given. When using these instruments the percentage must be multiplied by the standard for the particular instrument in order to obtain grams per 100 c.c.

When all investigators report their figures in grams per 100 c.c. of blood the results from all laboratories may be compared very easily without laboriously determining what instrument was used and upon what standard that instrument is calibrated. There is one precaution, however, that should be taken. All instruments should be standardized against Wong's⁴ iron method or Van Slyke's⁵ oxygen capacity method of determining hemoglobin. If all instruments are not properly calibrated figures will be of no more value than when the per cent of normal was used.

We have not space here to discuss the accuracy of the various methods of determining hemoglobin and it is unnecessary for both Robscheit⁶ and Senty⁷ have made excellent criticisms of the common methods in use, but we do wish to mention our experience in this laboratory. We have standardized our Von Fleischl-Miescher and Newcomer instrument with Wong's iron method and find them to check well within experimental error but in standardizing our Dare instrument we encounter the same difficulties reported by Lindsay, Rice, and Sellinger. We have therefore discarded the Dare in all our work except with our chicks. With them all readings are below 65 per cent and at such a low reading fairly accurate results are obtained. In this case, however, we make a definite note of the fact that the Dare instrument was used and hesitate to compare the figures with those obtained by the use of the other instruments.

SUMMARY

We suggest that all hemoglobin determinations be reported in grams per 100 c.c. of blood.

Reasons for the necessity of making this change and the value derived from using this standard method are given.

The need for proper standardization of instruments, and the precautions which should be taken in dealing with figures from an improperly calibrated instrument are pointed out.

REFERENCES

- ¹Editorial Column Jour Am Med Ass 1926 LXXVII 1323
²Williamson, C S Arch-Int Med, 1916, xviii 505
³Lindsay J W, Race, C, Selinger M A JOUR LAB AND CLIN MED 1926, xi, 737
⁴Wong San Yin Jour Biol Chem 1923 iv 421
 Van Slyke, D D Jour Biol Chem 1918, xxxiii 127
⁶Robscheit, F S Jour Biol Chem, 1920 xli 209
⁷Senty, E G JOUR LAB AND CLIN MED 1923 viii 591

FLOCCULATION TESTS IN THE SEROLOGY OF SYPHILIS

BY DR ERNST MLINCKE*

TRANSLATED BY A. MOLLHANN M.D., GRAND RAPIDS, MICHIGAN

UP TO 1917 all attempts have failed to improve or replace the Wassermann reaction with a flocculation reaction. None of the many recommended tests have been satisfactory in practice.¹ The causes of the failure were the unknown experimental elements for such reactions.

For the first time in 1917 I published a test for syphilis² and investigated systematically the experimental elements of the lues flocculation tests on the whole. I determined the properties of the organ extracts and the sera, and examined the influence of temperature of different salt solutions, the inactivation of the sera, the dosage of the reagents, etc.³ I succeeded in offering three different methods of lues diagnostics. The first method (Wasser methode) favors flocculation in negative sera only; the second method (Koch salzmethode) favors flocculation in all sera, the floccules dissolving upon addition of salt (sodium chloride) in the negative sera and staying in the positive sera. As third method I chose an arrangement which caused flocculation in the positive sera only.

It may be mentioned that I developed the 'Kochsalzmethode' to an immunity reaction in cooperation with Blev and Neumann.⁴ I used the method in the diagnosis of malleus (Glanders) of the horses with best result. To the alcoholic organ extract we added an antigen from *B. mallei* and mixed this with sera from infected and healthy horses. Flocculation was formed in the sera of the horses infected by malleus which stayed upon addition of sodium chloride, but the floccules in the sera of healthy horses dissolved upon addition of sodium chloride. The complex malleus antigen—malleus antibody—is proved by the fact that in analogy to the positive lues reaction there are formed floccules resistant to sodium chloride, probably due to a compound of the extract lipoids and serum bodies. I called the new immunity reaction "lipoid binding reaction". Dahmen⁵ used the method with best result for the diagnosis of trypanosomiasis equiperdum and the specific interstitial pneumonia of cattle. Denker⁶ used it for the diagnosis of the contagious stool of cattle. The "lipoid binding reaction" is equivalent to the agglutination or the complement deviation as specific of all contagious diseases and is specific differentiation of albumin. It is an immunity reaction sui generis.

After Sachs and Georghi¹⁰ convinced themselves of the correctness of the experimental elements of the flocculation reactions, which I elaborated and demonstrated, and published in 1918, this method has spread widely ever since under the name "Sachs-Georghi Reaction" (S G R). The S G R asks for cholesterinized extracts from cattle heart which Sachs used for the Wassermann reaction. The test is done in the medium of a physiologic salt solution. In 1919 I recommended for practical use the "third modification"¹¹ of my flocculation reaction which gives flocculation in the positive sera only. This method is usually called "D M" in numerous laboratories. It is characteristic for the "D M" that the extracts are obtained from horse heart which are extracted first by ether and then by alcohol. The reaction is done in the medium of a hypertonic salt solution.

Concerning the preparation of my extracts and the technic of the "D M" I refer to my previous publications.

Following the example of the "D M" and "S G R" all later lues reactions have been elaborated and recommended by a number of authors. The methods differ only in the choice of the organ extracts, in the variety of the additional agents, in the different salt content of the media, and the different ratio between the volume of serum and extract used. As far as the preparation of the extracts is concerned the previous extraction with ether or similar chemicals I recommended, has proved satisfactory. The addition of cholesterin, as recommended by Sachs, is frequently used to strengthen the extract, or both have been combined. Among the numerous authors, reactions have been recommended by Hecht, Bruck, Kodama, Vernes, Dreier, Waid, and Kahn. An improvement in the reactions has been attempted by reducing the amount of extract to a smaller volume in comparison to the amount of serum used, this pertains to the reactions recommended by Hohn¹⁴ and Kahn¹⁵.

In the meantime it has been made a particular study to convert the lues reaction into speed reactions (Dodd¹⁶). As is known a Wassermann reaction is completed within a few hours while the flocculation methods always asked for an entire day. This was a disadvantage, therefore I elaborated a speed reaction, the "Meinicke-Triubungsreaction" (M T R)^{17, 18, 19} which is characterized by the addition of balsam of Tolu to the organ extract. I chose balsam of Tolu because it appeared to me very suitable to improve the accuracy and speed of the reaction. Many improvements and changes in the flocculation tests have been advised, it is impossible to describe them all. None of them offer anything new in principle.

Generally speaking the following comments may be made from previous experiences:

1. None of the methods described hitherto will replace the Wassermann reaction entirely. There are cases of lues in which flocculation tests fail, and the Wassermann reaction only is positive.

2. According to the scientific opinions of today, it is not justified that laboratories operate the Wassermann reaction only. There are many cases of primary and latent lues where the Wassermann reactions fail, and the flocculation and precipitation tests are positive. At least one of the substitute methods should always be operated at the same time with the Wassermann reaction.

3 The accuracy of the reactions is greatly improved by operating different methods at the same time and the number of false readings are reduced to a minimum

PREPARATION OF THE EXTRACT

Horse heart is finely powdered and dried the powder is extracted first by ether then by alcohol, the degree of concentration of the ether rest extract is determined empirically, the extract is diluted with alcohol, 96 per cent, to about 1:14, then balsam of Tolu and benzoic acid are added. The extracts are to be kept at room temperature protected from light.

DILUTION OF THE EXTRACT

A quantity of extract sufficient for the number of tests intended is put into a tube, and ten times the quantity of a 3 per cent sodium chloride solution containing 0.01 per cent crystallized sodium carbonate in another tube. Both tubes are warmed in a water bath for five to ten minutes to a temperature of 45°, then the solutions are mixed rapidly by pouring the salt solution into the extract, the mixture is then poured back into the empty tube to insure thorough mixing.

SERA

The sera must be made perfectly clear by centrifugalization. They are not to be inactivated, but must be used active.

TECHNIC OF THE TEST

To each 0.2 cc serum is added 1.0 cc of the freshly prepared extract dilution, after the latter has ripened for a few minutes. The test tubes stay for one hour in a warm room (20° Celsius).

READING OF THE RESULTS

The examiner stands before a light window in a distance of about 2 to 3 meters, and watches the crossbar of the window through the reagents in the test tubes. In gloomy weather the readings are done by artificial light.¹⁰⁰ Similar to the x-ray plate illuminating cases a small case with two hidden bulbs may be used. The front side of the case is closed to an opening of 15 x 20 cm, into which is fastened a plate of opaque glass covered with a broad black cross. The reagents in the test tubes are examined by watching the cross in the glass through the light, holding the test tubes at about 60 cm distance from the case.

Reactions are negative if the transparency of the fluid is unimpaired. The cross has distinct straight lines and appears black. In strongly positive reactions the fluid is entirely opaque the cross cannot be seen. In weakly positive reactions the cross appears in grey color with indistinct contours, as if it were lying behind a veil.

In small laboratories where only few specimens are tested at one time or in weakly positive cases or if preferred for other reasons, a control may be used. Each specimen is run with two tubes, one for the test proper the

other one for the control. The only difference is the addition of one drop of formaldehyde, 40 per cent, in the control tube at the beginning of the test. If the main tube has the same appearance as the control tube, the specimen is negative. If the main tube is more opaque than the control tube the specimen is positive, the degree of positiveness depending upon the degree of opacity.

The young author, Dohnal, who lately died in Innsbruck, has converted my "Trübungsreaktion" into a microreaction.²¹ My assistant, Di Gross,² and I²⁰ have added further improvement to this new method, the "Meimcke Micra reaction" only in a somewhat weaker serologic adjustment. The dilution of the extract is done in the same manner as described above.

SERA

Sera must be active. Minute quantities of blood, as may be drawn into a capillary from a needle prick into the finger tip or the ear lobe, are sufficient for the test.

TECHNIC OF THE TEST

Mixing the diluted extract and the serum is best done by means of gauged platinum wire ring inoculating needles. After the fresh extract dilution has ripened for a few minutes it is poured into a small porcelain dish. By means of platinum needle with an eye of 5 mm diameter a drop of extract dilution is taken. By means of another loop with a diameter of only 2.5 mm a drop of serum is mixed within the lumen of the larger needle with the diluted extract. With the smaller loop a drop of the mixture is then placed upon a cover glass on a hollow slide, and left for three-fourths to one hour at a temperature of 20° to 22° C.

READING THE RESULTS

The drops are examined microscopically with a weak eyepiece and a strong dry objective (for instance Leitz eyepiece 1 or 2, and dry objective 6 or 7).

The different layers of the drop must be focalized and examined.

The reaction is negative if one sees either nothing or numerous exceedingly minute vivid particles in molecular movement. Strong positive reactions are characterized by the appearance of thick floccules which sink to the bottom layers of the drop due to their gravity, and become the larger the closer they lie to the bottom. In weak reactions the microscopic field is covered with more or less large floccules lying close to one another which are larger on the lower pole of the drop than in the upper layers.

Very elegant pictures are seen by examining the "hanging" drop in the dark-field as recommended by Hartwich.²³ If the necessary lenses are not available, one may examine a layer preparation in the dark-field instead of the "hanging" drop. A reaction is negative if one sees an immense number of uniformly distributed light dancing particles in a grey field. A reaction is positive if one sees scattered more or less large jagged brilliant white floccules.

cules in a deep black field. Between these findings there are all shades according to the intensity of the reactions.

The macroreaction (MTR) and the microreaction (MMR) are exceedingly convenient methods for the diagnosis of syphilis and offered to the free use of the profession. Every body who is acquainted with the use of a pipette and the simplest serologic technique can operate these methods. Reading the results does not require any more practical experience than the determination of albumin in the urine. The observation of the microreaction requires the knowledge of the usual microscopic technique only.

There is no need for an incubator and an exactly adjusted water bath for the inactivation of the sera. The microreaction can be done even without a centrifuge. If the blood specimens are collected in narrow glass tubes, they may be left overnight, while the serum will separate from the coagulum. By means of the eye of a platinum wire needle a minute quantity of the clear serum is obtained. To operate the MMR one needs only a few glass containers, a few platinum inoculating needles, and a microscope.

The elegance and simple technique of my methods have been recognized in the literature. I refer to the original articles of, for instance, Klopstock,⁴ "There are a large number of statements which speak in highest terms of the elegance and speed of the Mehncke Trubungs reactions. I further refer to the original articles of Untersteiner,⁵ Prochazka,⁶ Oro Augusto,⁷ Poschacher,⁸ Pais,⁹ Kirchner,¹⁰ Ivanoff,¹¹ Hager,¹² Petersen,¹³ Saunders,¹⁴ Szirmai,¹⁵ Lanbenheimer and Hamel,¹⁶ Van der Hoeden,¹⁷ Schilling,¹⁸ Tedeschi,¹⁹ Untersteiner,²⁰ Ruder,²¹ Strempel,²² and Westford.²³

Relative to the sensitiveness of my reactions Klopstock and Hilpert²⁴ make the following statement in their comprehensive article, "Most original articles agree on the fact that the Mehncke Trubungs Reactions have a high sensitiveness." For further information it may be referred to Delitala,²⁵ Panofsky,²⁶ Beretvas,²⁷ Mylius,²⁸ Klaffen,²⁹ Alexander and Emmich,³⁰ Bering,³¹ Fortig,³² Klem,³³ Kruspe,³⁴ de Benedetti,³⁵ Behrmann,³⁶ Schukri,³⁷ Fabian,³⁸ Richter,³⁹ Klopstock and Dolter,⁴⁰ Elkeles,⁴¹ and others.

According to my own experiences my methods agree in about 95 per cent of all cases with the Wassermann reaction. Of course the percentage is dependent upon the type of syphilitic patient examined. The more recent cases of lues, controls of treatment, and latent cases of lues are to be examined, the more frequent quantitative and qualitative differences will be observed in the use of different methods. But they never exceed 10 per cent according to my experiences. As far as different reacting syphilitic patients are concerned, the MTR and MMR are superior to the Wassermann reaction, in about two thirds of the cases in sensitiveness. The Wassermann reaction, in about only one third of the diverging cases with negative results of my in

cently admitted freely that the specificity of the M T R is preserved well. He states that the M T R in its recent form is a very valuable reaction for syphilis, and has great advantages over the other methods.

I especially quote Klopstoeck's statements, as they are based upon very large material, and originated from Saehs' Institute, where the SGR and the Benzoehol-reaction have been discovered, competitive methods of my reactions. The criticism of Saehs' school on my methods appears to me especially valuable.

The practical usefulness of the M T R and the M M R has been proved. For further information on the microreaction (M M R) I refer to Dohnal,⁷⁷ Niederwieser,⁷⁸ Spieca,⁷⁹ Loevy,⁸⁰ Martin,⁸¹ Hilgers and Kotzing,⁸² Hartwich,⁸³ Butschei,⁸⁴ Post,⁸⁵ and Peterson-Saunders.⁹² In 1925 the number of sera examined in the different laboratories by the M T R and M M R far exceeded one million.

The main advantage of my reactions, however, is their cheap and simple technique and the speed of their operation. I explained previously the disadvantages that are necessarily attached to any centralizing institute. The main disadvantage is the limitation of examinations to clinically suspected cases only, where anamnestic data have pointed to lues. Up to the present only a comparatively small number of patients have been examined for syphilis. Therefore numerous cases of syphilis were not diagnosed, and received the wrong treatment. Sources of infection were not detected, and gave occasion for further spreading of syphilis. Now every hospital, which has a doctor who is acquainted with the simple manipulations of laboratory technique, is able to examine systematically the entire material of patients for syphilis. The same opportunity is given to the private physician who is able to build up a small laboratory with small means.

I asked Koster,⁸⁶ and Koster and Amend⁸⁷ to publish the experiences that we obtained from a systematic examination for lues of all patients of a tuberculosis sanitarium. Not more than at the highest one-third to one-fourth of the syphilitic patients admitted to the sanitarium were clinically suspicious of syphilis. The other two-thirds to three-fourths were detected by systematic serologic examination only. Kohn von Jaski,⁸⁸ Hager,⁸⁹ and Eicke⁹⁰ had exactly the same experience in their tuberculosis sanitarium. The majority of their syphilitic patients were found out by systematic serologic examination only. Antiluetic treatment greatly improved the apparently serious cases of tuberculosis. All these authors emphasize the high specificity of the M T R, that always gave specific reactions regardless of the seriousness of the tuberculous cases examined.

Following these favorable experiences, a number of German government boards have recently ordered the systematic examination with my reactions on all patients of their hospitals and sanitariums. We are here at the beginning of a development that certainly will be followed by great results. The blood examination for syphilis should be done with same regularity as the examination of the urine for albumin and sugar. To create the methods for the systematic examinations for syphilis was my aim.

REFERENCES

- ¹Jacob thal Die Präzipitations und Flockungs Reaktionen zum Luessnachweis, Brucks Handb f Serodiagnose der Syph 1924
- Meincke Berl klin Wehnschr 1917 No 20 p 613
- ²Meincke (a) Ztschr f Immunitätsforsch u exper Therap 1918 xxvii, 350
 - (b) Ibid 1919 xxviii 280
 - (c) Ibid 1920 xxix 396
 - (d) Deutsch med Wehnchr 1919 Nos 24 and 51
 - (e) Ibid 1919 p 178
 - (f) Ibid 1920 No 1
 - (g) Munchen med Wehnchr 1917 pp 1644 and 1464
 - (h) Berl klin Wehnchr 1918 No 4
 - (i) Deutsch med Wehnchr 1919 p 32
 - (j) Ibid 1920 p 1022
 - (k) Ibid 1922 p 1132
 - (l) Ibid 1922 p 113
 - (m) Ztschr f arztl Fortbildung, 1921 p 156
 - (n) Ztschr f Immunitätsforsch u exper Therap 1918 xxvii 517
- ³Meincke Munchen med Wehnchr 1915 p 1379
- ⁴Lesser Munchen med. Wehnchr 1918 No 32
 - Deutsch med Wehnchr 1918 No 4.
- ⁵Meincke Berl klin Wehnchr 1917 p 108
- Meincke and Bley Ztschr f Veterinärk 1919 No 3
- ⁶Meincke and Neumann Ztschr f Veterinärk 1919 No 6
- Meincke Berl Tierarztl Wehnchr 1919 No 44
- ⁷Dahmen in Weichhardt Ergebnisse der Hygiene Bakteriologie
 - (a) Immunitätsforsch u exper Therap xi
 - (b) Berl Tierarztl Wehnchr 1921 No 7
 - (c) Arch f wissenschaftliche u prakt Tierh xliii No 5
- ⁸Denker Monatschr f prakt Tierh xxvi
- ⁹Sachs and Georgi Med Klin 1918 p 805
- ¹⁰Meincke Munchen med Wehnchr 1919 No 33
- ¹¹Sachs H Ztschr f Immunitätsforsch u exper Therap xl 149
- ¹²Meincke Munchen med Wehnchr 1920
- ¹³Hohn Munchen med Wehnchr 1920 No 51
 - Ibid 1925 No 11
- ¹⁴Kahn Serum Diagnosis of Syphilis by Precipitation Baltimore 1920 Williams and Wilkins Co (Monograph)
- ¹⁵Dodd Med Klin 1921 No 31
- ¹⁶Meincke Deutsch med Wehnchr 1922 No 1
- ¹⁷Sachs Klopstock and Chasch Klin Wehnchr 1924 No 30
- ¹⁸Meincke (a) Deutsch med Wehnchr 1923 Nos 2 and 19
 - (b) Klin Wehnchr 1924 No 9
 - (c) Munchen med Wehnchr 1924 No 17
 - (d) Deutsch. med. Wehnchr 1926
- ¹⁹Meincke Med Klin 1925 No 4
 - Dermat Wehnchr, 1925 No 26
- ²⁰Dohnal (a) Dermat Wehnchr 1923 No 34
 - (b) Klin Wehnchr 1925 No 10
 - (c) Dermat Wehnchr 1925 Nos 10 and 26
- ²¹Gross Dermat Wehnchr 1926
- ²²Hartwich Klin Wehnchr 1925, No 52
- ²³Klopstock A. Ergebnisse der neueren Did u Kinderh 1925 xxviii
 - Untersteiner Arch f Psychiat lxxii No 2
- ²⁴Prochazka Ceska Dermat xi No 2
- ²⁵Oro Foho med Gx No 21
- ²⁶Poschacher Dermat Wehnchr lxxix No 33
- ²⁷Pais Ann d mal vener xx No 4
- ²⁸Kirchner Orvosi hetilap lxi No 12
- ²⁹Ivanoff Rev gen d opht xxxviii No 11
- ³⁰Hager Ztschr f Tuberk xliii No 6
- ³¹Petersen Saunders Jour Lab and Clin Med 1926 vi, No 5
- ³²Szirmai Jahrb f Kinderh lv
- ³³Laubenheimer and Hamel Med Klin 1923 p 1612
- ³⁴van der Hoeven Tijdschr voor Geneeskunde 1923 i No 7
- ³⁵Schilling Deutsch med Wehnchr 1924 i No 25
- ³⁶Tedeschi Gior ital d mal ven e d pelle lvi No 2
- ³⁷Untersteiner Deutsch med Wehnchr 1924 No 13
- ³⁸Röder Dermat Wehnchr lxxviii Nos 13 and 14

- 41Stiempel Dermat Wehnschr, xxxv, No 6, p 1023
- 42Sachs and Rondoni Berl klin Wehnschr, 1908, p 1968
- 43Klopstock and Hilpert Klin. Wehnschr, 1926, No 7
- 44Dehtala Studi sassaresi, 1925, I, No 1
- 45Panofsky Dermat Wehnschr, lxxxi, No 45
- 46Beretvas Polichinico, sez med, 1925, xxxii, No 9
- 47Mylius Fortschr med, vi, Nos 11 and 12
- 48Klaften Zentralbl f Gynak, 1925, No 1
- 49Alexander and Emmich Med Klin, 1925, No 8
- 50Bering Centralbl f Bakteriolo, 1923, vi
- 51Fortig Deutsch med Wehnschr, 1923, No 6
- 52Klein Deutsch med Wehnschr, 1923, No 8
- 53Kruspe Dermat Wehnschr, 1925, No 39
- 54Do Benedetti Gaz delgi Ospedali e d clin, 1925, No 31
- 55Behrmann Dermat Ztschr, 1925, viii, Nos 1 and 2
- 56Schukri Munchen med Wehnschr, 1925, No 5
- 57Fribian Bratislavske lekarske listy, 1924, No 3
- 58Richter Deutsch med Wehnschr, 1924, No 14
- 59Klopstock and Dolter Klin Wehnschr, 1924, No 23
- 60Elkeles Deutsch med Wehnschr, 1924, No 23
- 61Fortig Munchen med Wehnschr, 1924, No 12
- 62Pomini and Cascella
- 63Schultze Klin Wehnschr, 1924, No 14
- 64Kruchen Deutsch med Wehnschr, 1924, No 2
- 65Klopstock and Hilpert Klin Wehnschr, 1926, No 9
- 66Boas Arch f Dermat, 1924, p 443
- 67Jaenecke Deutsch med Wehnschr, 1923, p 1568
- 68Sato Med Klin., 1923, p 1160
- 69Schubert Deutsch med Wehnschr, 1923, p 352
- 70Untersteiner Dermat Wehnschr, 1924, p 1042
- 71Hilgers Ztschr f Immunitatsforsch u exper Therap, 1924, vi, No 2
- 72Elkeles Ztschr f Immunitatsforsch u exper Therap, 1924, xxxv, No 4
- 73Mutermilch and Compagnon Compt rend Soc de biol, 1925, xcii, No 3
- 74Austernweil Gyogyszat, 1925, No 30
- 75Rocca Polichinico, sez med, 1925, xxxii, No 6
- 76Knebel Zentralbl f Gynak, 1925, No 24
- 77Dohnal Dermat Wehnschr, 1923, No 34
- Dermat Wehnschr, 1925, Nos 10 and 26
- 78Niederwieser Wien klin Wehnschr, 1924, No 39
- 79Spicca Polichinico, sez priat, 1925, xxxii, No 43
- 80Loewy Monatschr f Kinderh, 1925, xxx
- 81Martin Deutsch med Wehnschr, 1925, No 43
- 82Hilgers and Kotzing Med Klin, 1925, No 43
- 83Hartwich Klin Wehnschr, 1926, No 5
- 84Bartscher Munchen med Wehnschr, 1926, No 5
- 85Post Dermat Ztschr, vi, Nos 5 and 6
- 86Koster Ztschr f Tuberk, xxxviii, No 2
- 87Koster and Amend Ztschr f Tuberk, vi, No 6
- 88Kohn von Jaski Ztschr f Tuberk, viii, No 4
- 89Hager Ztschr f Tuberk, viii, No 6
- 90Eicke Ztschr f Tuberk, viii, No 3
- 91Mufford Ill Med Jour, 1926, No 1
- 92Petersen Saunders Arch Path Lab Med, 1926, No 1

TABLES FOR CALCULATION OF COLOR INDEX, VOLUME INDEX AND SATURATION INDEX BASED ON RECENTLY DETERMINED STANDARDS*

By EDWIN E. OSGOOD, M.D., PORTLAND, OREGON

REDETERMINATIONS of the hemoglobin red cell count cell volume color index, volume index and saturation index standards have shown that the figures generally given in the texts are incorrect, and a study of the literature reveals that they have never had a very sound experimental basis. Obviously, the newer figures, based on the examination of the bloods of 137 healthy young men¹ and 100 healthy young women by the most accurate methods available, should be used in all index calculations until study of a larger series of cases can give us still more accurate figures. While these standards are based on examinations of young adults experience has shown that no great error is involved in using them for older individuals until further work establishes definite standards for these ages.

Table II is so designed that the results of blood studies can be converted into terms of per cent of the new standards without calculation and Chart I is so constructed that the various indexes may be found from these data by inspection without calculation.

DISCUSSION OF THE STANDARDS ON WHICH TABLE II IS BASED

Accurate red cell counts hemoglobin estimations and cell volume determinations on the oxalated venous blood of 137 healthy young men and 100 healthy young women gave the results in Table I.

TABLE I

RED CELLS MILLION PER CMM			HEMOGLOBIN GM PER 100 CC		CELL VOLUME CC PACKED CELLS IN 100 CC OF BLOOD	
	Average	Results in 90% of the cases	Average	Results in 90% of the cases	Average	Results in 90% of the cases
Men	5.4	47.61	15.8	140.190	45	40.50
Women	4.8	43.53	13.7	120.155	41	37.45

We retain the use of five million as 100 per cent red cells in the index determinations simply for convenience in calculation. Any other figure would do, if it were generally agreed upon. Of course, five million is not the average red cell count for either normal men or women. If five million is taken as 100 per cent red cells, then the average hemoglobin coefficient (a term introduced by us for the number of grams of hemoglobin per 100 cc of blood calculated to a red cell count of five million) must be taken as 100 per cent

*From the Department of Biochemistry, University of Oregon Medical School
Received for publication December 7, 1926

TABLE II

1	2	3 MEN	4	5 WOMEN	6	7
PER CENT	RED CELL COUNT, MILLIONS PER C MM	HEMOGLOBIN, GRAMS PER 100 CC	VOLUME OF PACKED CELLS, CC PER 100 CC	HEMOGLOBIN, GRAMS PER 100 CC	VOLUME OF PACKED CELLS, CC PER 100 CC	HEMOGLOBIN, GRAMS PER 100 CC
10	0.50	1.47	4.10	1.43	4.70	1.38
11	0.55	1.62	4.51	1.57	4.73	1.52
12	0.60	1.76	4.92	1.71	5.16	1.66
13	0.65	1.91	5.33	1.86	5.59	1.79
14	0.70	2.06	5.74	2.00	6.02	1.93
15	0.75	2.21	6.15	2.15	6.45	2.07
16	0.80	2.35	6.56	2.29	6.88	2.21
17	0.85	2.50	6.97	2.43	7.31	2.35
18	0.90	2.65	7.38	2.57	7.74	2.48
19	0.95	2.79	7.79	2.72	8.17	2.62
20	1.00	2.94	8.20	2.86	8.60	2.76
21	1.05	3.09	8.61	3.00	9.03	2.90
22	1.10	3.23	9.02	3.15	9.46	3.04
23	1.15	3.38	9.43	3.29	9.89	3.17
24	1.20	3.53	9.84	3.43	10.32	3.31
25	1.25	3.67	10.25	3.58	10.75	3.45
26	1.30	3.82	10.66	3.72	11.18	3.59
27	1.35	3.97	11.07	3.86	11.61	3.73
28	1.40	4.12	11.48	4.00	12.04	3.86
29	1.45	4.26	11.89	4.15	12.47	4.00
30	1.50	4.41	12.30	4.29	12.90	4.14
31	1.55	4.56	12.71	4.43	13.33	4.28
32	1.60	4.70	13.12	4.58	13.76	4.42
33	1.65	4.85	13.53	4.72	14.19	4.55
34	1.70	5.00	13.94	4.86	14.62	4.69
35	1.75	5.15	14.35	5.00	15.05	4.83
36	1.80	5.29	14.76	5.15	15.48	4.97
37	1.85	5.44	15.17	5.29	15.91	5.11
38	1.90	5.59	15.58	5.43	16.34	5.24
39	1.95	5.73	15.99	5.58	16.77	5.38
40	2.00	5.88	16.40	5.72	17.20	5.52
41	2.05	6.03	16.81	5.86	17.63	5.66
42	2.10	6.17	17.22	6.01	18.06	5.80
43	2.15	6.32	17.63	6.15	18.49	5.93
44	2.20	6.47	18.04	6.29	18.92	6.07
45	2.25	6.62	18.45	6.44	19.35	6.21
46	2.30	6.76	18.86	6.58	19.78	6.35
47	2.35	6.91	19.27	6.72	20.21	6.49
48	2.40	7.06	19.68	6.86	20.64	6.62
49	2.45	7.20	20.09	7.01	21.07	6.76
50	2.50	7.35	20.50	7.15	21.50	6.90
51	2.55	7.50	20.91	7.29	21.93	7.04
52	2.60	7.64	21.32	7.44	22.36	7.18
53	2.65	7.79	21.73	7.58	22.79	7.31
54	2.70	7.94	22.14	7.72	23.22	7.45
55	2.75	8.08	22.55	7.87	23.65	7.59
56	2.80	8.23	22.96	8.01	24.08	7.73
57	2.85	8.38	23.37	8.15	24.51	7.87
58	2.90	8.53	23.78	8.29	24.94	8.00
59	2.95	8.67	24.19	8.44	25.37	8.14
60	3.00	8.82	24.60	8.58	25.80	8.28
61	3.05	8.97	25.01	8.72	26.23	8.42
62	3.10	9.11	25.42	8.87	26.66	8.56
63	3.15	9.26	25.83	9.01	27.09	8.69
64	3.20	9.41	26.24	9.15	27.52	8.83
65	3.25	9.55	26.65	9.30	27.95	8.97
66	3.30	9.70	27.06	9.44	28.38	9.11
67	3.35	9.85	27.47	9.58	28.81	9.25
68	3.40	10.00	27.88	9.72	29.24	9.38
69	3.45	10.14	28.29	9.87	29.67	9.52

TABLE II—CONT D

1	2	3 MEN		5 WOMEN		7
		HEMOGLOBIN GRAMS PER 100 C C	VOLUME OF PACKED CELLS C C PER 100 C C	HEMOGLOBIN GRAMS PER 100 C C	VOLUME OF PACKED CELLS, C C PER 100 C C	
70	3.50	10.1	29.10	10.01	0.10	9.66
71	3.55	10.44	29.11	10.15	30.53	9.80
72	3.60	10.58	29.52	10.30	50.96	9.94
73	3.65	10.7	29.91	10.44	31.39	10.07
74	3.70	10.88	50.34	10.58	31.52	10.21
75	3.75	11.03	30.75	10.73	52.25	10.35
76	3.80	11.17	31.16	10.87	32.68	10.49
77	3.85	11.52	31.5	11.01†	3.11	10.63
78	3.90	11.47	51.98	11.15	3.54	10.76
79	3.95	11.61	32.39	11.30	33.97	10.90
80	4.00	11.76	52.50	11.44	4.40	11.04
81	4.05†	11.11	33.21	11.58	4.53	11.18
82	4.10	12.05	3.62	11.73	35.26†	11.32
83	4.15	12.20	34.03	11.87	5.69	11.45
84	4.20	12.35	34.44	12.01	36.12	11.59
85	4.25	12.49	34.85	12.16	36.55	11.73
86	4.30	12.64	52.1	12.30	36.98	11.87
87	4.35	12.79	52.1	12.44	37.41	12.01
88	4.40*	12.94	36.08	12.58	37.84	12.14
89	4.45	13.08	36.49	12.73	38.27	12.28
90	4.50	13.23	36.90	12.87	38.70	12.42
91	4.55	13.34	37.1	13.01	39.13	12.56
92	4.60	13.52	37.72	13.16	39.56	12.70
93	4.65	13.67	38.13	13.30	39.99	12.83
94	4.70	13.82	38.54	13.44	40.42	12.97
95	4.75	13.96	38.95	13.59	40.85	13.11
96	4.80††	14.11	39.36	13.73††	41.28††	13.25
97	4.85	14.26	39.77	13.87	41.71	13.39
98	4.90	14.41	40.18	14.01	42.14	13.52
99	4.95	14.55	40.59	14.15	42.57	13.66
100	5.00	14.70	41.00	14.30	43.00	13.80
101	5.05	14.85	41.41	14.44	43.43	13.94
102	5.10	14.99	41.82	14.59	43.86	14.08
103	5.15	15.14	42.23	14.73	44.29	14.21
104	5.20	15.29	42.64	14.87	44.72	14.35
105	5.25	15.44	43.05	15.01	45.15	14.49
106	5.30	15.58	43.46	15.16	45.58	14.63
107	5.35	15.73	43.87	15.30	46.01†	14.77
108	5.40	15.88	44.28	15.44	46.44	14.90
109	5.45	16.02	44.69	15.59	46.87	15.04
110	5.50	16.17	45.10	15.73	47.30	15.18
111	5.55†	16.32	45.51	15.87	47.73	15.32
112	5.60	16.46	45.92	16.02		15.46
113	5.65	16.61	46.33	16.16		
114	5.70	16.76	46.74	16.30		
115	5.75	16.90	47.15	16.44†		
116	5.80	17.05	47.56	16.58		
117	5.85	17.20	47.97			
118	5.90	17.35	48.38			
119	5.95	17.49	48.79			
120	6.00	17.64	49.20			
121	6.05	17.79	49.61			
122	6.10	17.93	50.02			
123	6.15	18.08	50.43			
124	6.20	18.22	50.84			
125	6.25	18.37	51.25			
126	6.30	18.52	51.66			
127	6.35	18.67	52.07			
128	6.40	18.82	52.48			
129	6.45	18.96*	52.89			

worked out yet, but apparently it is never high. It is low in anemias due to chronic blood loss even if they complicate pernicious anemia, and is normal in most other anemias including uncomplicated pernicious anemia.

In the cases we have studied 90 per cent of color, volume and saturation indexes in normal individuals have fallen between 0.9 and 1.1 and indexes below 0.8 and over 1.2 have been pathologically significant.

EXPLANATION OF TABLE II

Column 1 is to be read as per cent. The red cell counts in column 2 are arranged on the basis of five million as 100 per cent, so that after finding the patient's count in this column, reference to the corresponding figure in column 1 gives the red cell count expressed as per cent of five million. The hemoglobin figure in column 3 is so calculated that after finding the man's hemoglobin figure expressed in grams per 100 cc in this column reference to column 1 will give the hemoglobin expressed as per cent of 14.7 gm, which is the normal hemoglobin coefficient in men.

Column 4 is so calculated that after finding the man's volume of packed red cells expressed as cc per 100 cc of blood in this column, reference to column 1 will give the cell volume expressed as per cent of 41 cc, which is the normal volume coefficient in men. The author's technique¹ for cell volume determination must be used.²

Columns 5 and 6 for women are similar to columns 3 and 4 for men but are based on the normal hemoglobin coefficient for women (14.3 gm) and the normal volume coefficient for women (43 cc). Column 7 is inserted for convenience in calculating the grams of hemoglobin in 100 cc of blood when the method used for estimation is based on a content of 13.8 gm per 100 cc as 100 per cent.

It is recommended that hemoglobin results be always reported in grams per 100 cc because so many different figures have been used as 100 per cent hemoglobin by manufacturers of hemoglobinometers that per cent figures are almost meaningless. We must repeat our warning³ that, although they are supposedly standardized so that 100 per cent is equivalent to 13.8 gm of hemoglobin, the Dare and Tallqvist methods are not sufficiently accurate for color index determinations.⁴

For hemoglobin methods in which 100 per cent is not exactly equivalent to 13.8 gm per 100 cc column 7 must, of course, be recalculated. The number of grams of hemoglobin per 100 cc of blood equivalent to an esti-

*This technique in brief is as follows: about 4 cc of oxalated (20 mg powdered potassium oxalate per 10 cc of blood) venous blood is centrifuged twenty to thirty minutes at high speed (over 3,000 revolutions per minute) in the special tube described below. The total volume of blood and the volume of cells is then noted and the blood recentrifuged for periods of at least five minutes until the volume of packed cells ceases to change. The cc of packed cells per 100 cc of blood is then calculated. The special tube is made by sealing the tip of a 10 cc Mohr pipette (graduated for 0.1 cc to the tip) cutting it off above the 6 cc mark (capacity 4 cc) and testing it for capacity at each 0.1 cc mark. Because of the small diameter of this tube it must be supported as follows: a cork of such diameter that it will rest on the bottom of the metal centrifuge cup is hollowed out to receive the tip of the special tube. A second cork slightly larger in diameter than the metal cup is so cut that it fits partly into it but is prevented by a lip from slipping entirely into the cup. This cork is then bored to fit snugly around the special tube and so steady it at the top.

†The methods of Van Slyke (J Biol Chem 33:127, 1918), Cohen and Smith (J Biol Chem 39:489, 1919), Haskins (J Biol Chem 57:111, 1923) and Osgood and Haskins (J Biol Chem 57:107, 1923) have been tested and found to be accurate although only the last three are clinically practical while the last two have the advantage of utilizing permanent standards. In all of the methods mentioned above an estimation of 100 per cent indicates a content of 13.8 gm of hemoglobin per 100 cc of blood.

mation of 100 per cent with the particular hemoglobinometer used must be placed in column 7 opposite 100 per cent in column 1, and other figures placed to correspond to this value

EXPLANATION OF THE CHART

Chart 1 is so designed that the vertical line corresponding to the intersection of any two printed lines of the logarithmic paper gives the quotient of the value indicated by the figure in the right hand column (X) divided by the value indicated by the figure in the left hand column (Y). Hence it can be used for the determination of all of the indexes if one simply remembers to always look up the numerator of the fraction expressing the index in the right hand column (X) and the denominator in the left hand column (Y).

EXAMPLE OF THE CALCULATION

The study of the blood of Mrs. F gave the following results

Red blood cells 162 million

Hemoglobin 52.0 per cent (Hanks-Sahli's method)

Volume of packed red cells 18.89 cc per 100 cc of blood (by the author's method)

Reference to column 2 (Table II) shows that this red cell count corresponds to 32 per cent (column 1) of 50 million red cells

Reference to columns 1 and 7 shows that an estimation of 52.0 per cent by this method is equivalent to 7.18 gm of hemoglobin per 100 cc of blood

Then looking up 7.18 in column 5 (the patient is a woman) we find that it is 50 per cent of the normal hemoglobin coefficient for women

In the same manner looking up 18.89 cc in column 6 we find that it is 44 per cent of the normal volume coefficient for women

Now we can determine the indexes by use of Chart 1. The color index is $\frac{50 (X)}{32 (Y)}$

Therefore, look up line 50 in column X and line 32 in column Y. We find that they intersect about midway between vertical lines 1.5 and 1.6 corresponding to a color index of 1.55

Similarly the volume index is $\frac{44 (X)}{32 (Y)}$. These lines intersect at a vertical line corresponding to a volume of 1.36

The saturation index is $\frac{44 (X)}{50 (Y)}$ which from Chart 1 is found to be 1.14

The laboratory report on this case would then read

Red blood cells 162 million

Hemoglobin 7.2 grams

Color index 1.55

Volume index 1.36

Saturation index 1.14

The high volume and color indexes with normal saturation index is pathognomonic of pernicious anemia

SUMMARY

Table II in this paper enables one to convert the results of red cell counts, hemoglobin estimations, and cell volume determinations directly into all the percentage figures that are necessary for the calculation of the color, volume and saturation indexes. The table can also be used to convert percentage of

The letters (X) and (Y) refer to the columns in Chart 1 in which the corresponding numerals should be looked up

TABLE II—CONT'D

1		2		3 MEN		4		5 WOMEN		6		7
PER CENT	PED CELL COUNT, MILLIONS PER C MM	HEMOGLOBIN, GRAMS PER 100 c c	VOLUME OF PACKED CELLS, c c PER 100 c c	HEMOGLOBIN, GRAMS PER 100 c c	VOLUME OF PACKED CELLS, c c PER 100 c c	HEMOGLOBIN, GRAMS PER 100 c c	VOLUME OF PACKED CELLS, c c PER 100 c c	HEMOGLOBIN, GRAMS PER 100 c c	VOLUME OF PACKED CELLS, c c PER 100 c c	HEMOGLOBIN, GRAMS PER 100 c c	VOLUME OF PACKED CELLS, c c PER 100 c c	HEMOGLOBIN, GRAMS PER 100 c c
130	6 50	19 11	53 30	18 59	55 90	17 94						
131	6 55	19 26	53 71	18 73	56 33	18 08						
132	6 60	19 40	54 12	18 88	56 76	18 22						
133	6 65	19 55	54 53	19 02	57 19	18 35						
134	6 70	19 70	54 94	19 16	57 62	18 49						
135	6 75	19 84	55 35	19 30	58 05	18 63						
136	6 80	19 99	55 76	19 45	58 48	18 77						
137	6 85	20 14	56 17	19 59	58 91	18 91						
138	6 90	20 29	56 58	19 73	59 34	19 04						
139	6 95	20 43	56 99	19 88	59 77	19 18						
140	7 00	20 58	57 40	20 02	60 20	19 32						
141	7 05	20 73	57 81	20 16	60 63	19 46						
142	7 10	20 87	58 22	20 31	61 06	19 60						
143	7 15	21 02	58 63	20 45	61 49	19 73						
144	7 20	21 17	59 04	20 59	61 92	19 87						
145	7 25	21 31	59 45	20 73	62 35	20 01						
146	7 30	21 46	59 86	20 88	62 78	20 15						
147	7 35	21 61	60 27	21 02	63 21	20 29						
148	7 40	21 76	60 68	21 16	63 64	20 42						
149	7 45	21 90	61 09	21 31	64 07	20 56						
150	7 50	22 05	61 50	21 45	64 50	20 70						

The average figures for red cell count hemoglobin estimation and volume of packed red cells in the series of healthy men that we recently reported¹ are indicated in the table by * * and the lowest and highest values found are indicated by †. Similarly the averages and extremes of variation found in the study of the bloods of 100 healthy young women are indicated by †† and † respectively.

hemoglobin in index calculations. Hence we use 14.7, the average hemoglobin coefficient for normal men as calculated from the above data, as 100 per cent hemoglobin in calculating indexes for men (column 3 in Table II), and 14.3, the average hemoglobin coefficient similarly calculated for women, as 100 per cent hemoglobin in calculating indexes for women (column 5 in Table II). In like manner we use 41, the average *volume coefficient* (defined by us as the c c of packed red cells per 100 c c of blood calculated to a red cell count of five million) for normal men, as 100 per cent cell-volume in calculating indexes for men (column 4 in Table II), and 43, the average volume coefficient for normal women, similarly calculated from our data, as 100 per cent cell-volume in computing indexes for women (column 6 in Table II).

The color index expresses the ratio of the hemoglobin per unit number of cells in the patient's blood to the average hemoglobin per unit number of cells in the blood of normal persons of the patient's sex and age group. It is $\frac{\% \text{ hemoglobin}}{\% \text{ red cells}}$. This index is high in uncomplicated pernicious anemia, but low in chlorosis and in anemias due to chronic blood loss, and within normal limits in most other anemias.

The volume index expresses the ratio of the mean size of the cells in the blood examined to the mean size of the cells in the average blood of normal individuals of the patient's sex and age group. It is $\frac{\% \text{ cell-volume}}{\% \text{ red cells}}$. It is high

in all cases of pernicious anemia, and low in anemias due to chronic blood loss and probably also in chlorosis (although this has not yet been sufficiently tested)

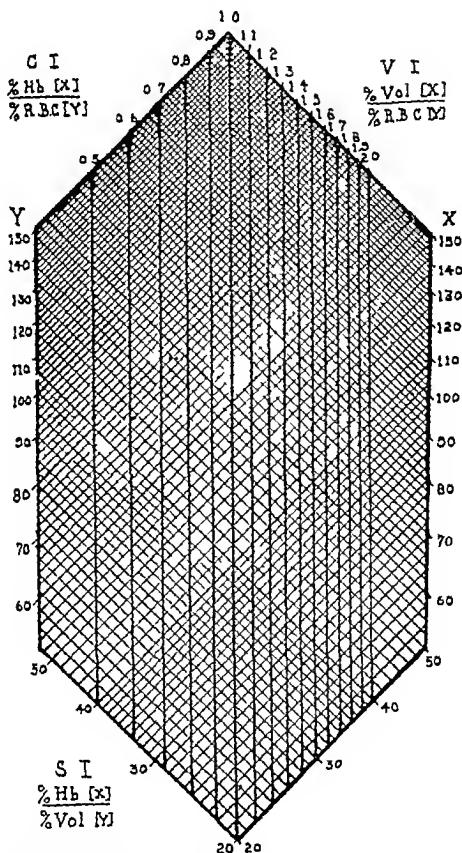


Chart 1—C I = color index. V I = volume index. S I = saturation index. If either percentage figure is less than 20 double both figures before reading the index from the chart. Indexes of 1 or more and 0.8 or less are definitely pathologic.

The saturation index expresses the ratio between the hemoglobin per unit volume of cells in the blood examined and the average hemoglobin per unit volume of cells in the blood of healthy persons of the same sex and in the same age group. It is $\frac{\% \text{ hemoglobin}}{\% \text{ cell volume}}$. Its significance has not been fully

worked out yet, but apparently it is never high. It is low in anemias due to chronic blood loss even if they complicate pernicious anemia, and is normal in most other anemias including uncomplicated pernicious anemia.

In the cases we have studied 90 per cent of color, volume and saturation indexes in normal individuals have fallen between 0.9 and 1.1 and indexes below 0.8 and over 1.2 have been pathologically significant.

EXPLANATION OF TABLE II

Column 1 is to be read as per cent. The red cell counts in column 2 are arranged on the basis of five million as 100 per cent, so that after finding the patient's count in this column, reference to the corresponding figure in column 1 gives the red cell count expressed as per cent of five million. The hemoglobin figure in column 3 is so calculated that after finding the man's hemoglobin figure expressed in grams per 100 cc in this column reference to column 1 will give the hemoglobin expressed as per cent of 14.7 gm, which is the normal hemoglobin coefficient in men.

Column 4 is so calculated that after finding the man's volume of packed red cells expressed as cc per 100 cc of blood in this column, reference to column 1 will give the cell volume expressed as per cent of 41 cc, which is the normal volume coefficient in men. The author's technique¹ for cell volume determination must be used.²

Columns 5 and 6 for women are similar to columns 3 and 4 for men but are based on the normal hemoglobin coefficient for women (14.3 gm) and the normal volume coefficient for women (43 cc). Column 7 is inserted for convenience in calculating the grams of hemoglobin in 100 cc of blood when the method used for estimation is based on a content of 13.8 gm per 100 cc as 100 per cent.

It is recommended that hemoglobin results be always reported in grams per 100 cc because so many different figures have been used as 100 per cent hemoglobin by manufacturers of hemoglobinometers that per cent figures are almost meaningless. We must repeat our warning³ that, although they are supposedly standardized so that 100 per cent is equivalent to 13.8 gm of hemoglobin, the Dare and Tallqvist methods are not sufficiently accurate for color index determinations.[†]

For hemoglobin methods in which 100 per cent is not exactly equivalent to 13.8 gm per 100 cc column 7 must, of course, be recalculated. The number of grams of hemoglobin per 100 cc of blood equivalent to an esti-

^{*}This technique in brief is as follows: about 4 cc of oxalated (20 mg powdered potassium oxalate per 10 cc of blood) venous blood is centrifuged twenty to thirty minutes at high speed (over 3,000 revolutions per minute) in the special tube described below. The total volume of blood and the volume of cells is then noted and the blood recentrifuged for periods of at least five minutes until the volume of packed cells ceases to change. The cc of packed cells per 100 cc of blood is then calculated. The special tube is made by sealing the tip of a 10 cc Mohr pipette (graduated for 0.1 cc to the tip) cutting it off above the 6 cc mark (capacity 4 cc) and testing it for capacity at each 0.1 cc mark. Because of the small diameter of this tube it must be supported as follows: a cork of such diameter that it will rest on the bottom of the metal centrifuge cup is hollowed out to receive the tip of the special tube. A second cork slightly larger in diameter than the metal cup is so cut that it fits partly into it but is prevented by a lip from slipping entirely into the cup. This cork is then bored to fit snugly around the special tube and so steady it at the top.

[†]The methods of Van Slyke (J Biol Chem 33:127, 1918), Cohen and Smith (J Biol Chem 39:489, 1919), Haskins (J Biol Chem 57:111, 1923) and Osgood and Haskins (J Biol Chem 57:107, 1923) have been tested and found to be accurate although only the last three are clinically practical while the last two have the advantage of utilizing permanent standards. In all of the methods mentioned above an estimation of 100 per cent indicates a content of 13.8 gm of hemoglobin per 100 cc of blood.

mation of 100 per cent with the particular hemoglobinometer used must be placed in column 7 opposite 100 per cent in column 1 and other figures placed to correspond to this value

EXPLANATION OF THE CHART

Chart 1 is so designed that the vertical line corresponding to the intersection of any two printed lines of the logarithmic paper gives the quotient of the value indicated by the figure in the right hand column (X) divided by the value indicated by the figure in the left hand column (Y). Hence, it can be used for the determination of all of the indexes if one simply remembers to always look up the numerator of the fraction expressing the index in the right hand column (X) and the denominator in the left hand column (Y).

EXAMPLE OF THE CALCULATION

The study of the blood of Mrs F gave the following results

Red blood cells 1 62 million

Hemoglobin 52 0 per cent (Haskins Sahli's method)

Volume of packed red cells 18 89 cc per 100 cc of blood (by the author's technique)

Reference to column 2 (Table II) shows that this red cell count corresponds to 32 per cent (column 1) of 5 0 million red cells

Reference to columns 1 and 7 shows that an estimation of 52 0 per cent by this method is equivalent to 7 18 gm of hemoglobin per 100 cc of blood

Then looking up 7 18 in column 5 (the patient is a woman) we find that it is 50 per cent of the normal hemoglobin coefficient for women

In the same manner looking up 18 89 cc in column 6 we find that it is 44 per cent of the normal volume coefficient for women

Now we can determine the indexes by use of Chart 1. The color index is $\frac{50 (X)}{32 (Y)}$. Therefore, look up line 50 in column X and line 32 in column Y. We find that they intersect about midway between vertical lines 1 5 and 1 6 corresponding to a color index of 1 55

Similarly the volume index is $\frac{44 (X)}{32 (Y)}$. These lines intersect at a vertical line corresponding to a volume of 1 36

The saturation index is $\frac{44 (X)}{50 (Y)}$ which from Chart 1 is found to be 1 14

The laboratory report on this case would then read

Red blood cells 1 62 million

Hemoglobin 7 2 gram

Color index 1 55

Volume index 1 36

Saturation index 1 14

The high volume and color indexes with normal saturation index is pathognomonic of pernicious anemia.

SUMMARY

Table II in this paper enables one to convert the results of red cell counts, hemoglobin estimations, and cell volume determinations directly into all the percentage figures that are necessary for the calculation of the color, volume and saturation indexes. The table can also be used to convert percentage of

The letters (X) and (Y) refer to the columns in Chart 1 in which the corresponding numerals should be looked up

hemoglobin (when estimated by methods in which 100 per cent indicates a content of 13.8 gm per 100 cc) into grams of hemoglobin per 100 cc of blood, thus enabling clinicians to report their hemoglobin findings in grams as has recently been strongly recommended.

In addition, the table shows the average figure and the significant variations for red cell counts, hemoglobin estimations, and cell-volume determinations in young men and women.

Chart 1 is so constructed that the color index, volume index and saturation index figures for any blood may be found by inspection without calculation from the percentage figures that have been obtained from Table II.

REFERENCES

- ¹Osgood, E. E. Hemoglobin, Color Index, Saturation Index, and Volume Index Standards, *Arch. Int. Med.*, May, 1926, *xxxvi*, 685.
- Osgood, E. E., and Haskins, H. D. The Relation Between Cell Count, Cell Volume, and Hemoglobin Content of the Venous Blood of Normal Young Women.
- ²Haskins, H. D. A New Permanent Standard for Sahli's Hemoglobinometer, *Jour. Biol.* September, 1926, *xxv*, 500.
- ³Haskins, H. D. A New Permanent Standard for Sahli's Hemoglobinometer, *Jour. Biol. Chem.*, August, 1927, *lxxviii*, 57.

A METHOD FOR CAPILLARY BLOOD SUGAR^{*}

By H. V. GIBSON, M.D., MADISON, WISCONSIN

THERE have recently been published a number of capillary blood sugar methods but we have been unable to use any of them satisfactorily in clinical work because of the quantity of blood required, special apparatus needed, or inaccurate results obtained. The following method represents the technic I adopted after considerable experimentation. It requires no unusual apparatus and involves but one micro measurement—that of the original blood sample. The maximum error found for the method has been 3 per cent when checked independently in three separate laboratories.[†] The method is a modification of the widely used Folin-Wu technic.¹

THE METHOD

Apparatus—1. A capillary pipette graduated to contain 0.1 cc.

2. Serology test tubes, of 4 cc. capacity with stoppers, one tube for each sample.

3. Folin-Wu sugar tubes, calibrated at 10 cc.

4. Lancet for capillary puncture.

Solutions—1. 0.115 N H₂SO₄.

2. 3 1/3 per cent sodium tungstate.

^{*}From the Laboratories of the Wisconsin Psychiatric Institute, University of Wisconsin and the Obstetric Department, Washington University School of Medicine, St. Louis, Mo.

Received for publication December 30, 1926.

[†]Chemistry Laboratory of Wisconsin General Hospital, Biochemistry Department Laboratory and the author's laboratory, all of University of Wisconsin.

3 Alkaline copper and phospho molybdate solution for Folin Wu method

4 Water, alcohol and ether for cleaning and drying pipette

5 Standards = 0.04 and 0.05 mg glucose per cc in saturated benzoic acid solution

Procedure—As many 4 cc tubes as the number of samples expected are racked, 1.9 cc of 0.115 N H_2SO_4 placed in each and the lot stoppered. Next, 0.1 cc blood is obtained from a puncture wound of the ear or finger tip and rinsed into the 0.115 N H_2SO_4 by alternately sucking up and blowing out. The tube is then stoppered, inverted to mix and set aside until the experiment is over. When all the tubes are so prepared, 1 cc of 3 1/3% sodium tungstate solution is added to each. The tube is then stoppered, shaken and centrifuged at high speed for ten minutes. Two cc of the supernatant fluid are then pipetted off and placed in one of the Folin Wu sugar tubes. Standards containing 0.8 and 0.1 mg glucose in 2 cc are suitable for all ordinary work. (Such standards keep for weeks if made up in half saturated benzoic acid solutions.)

The alkaline copper reagent and the digestion and color development carried out exactly as in the macro Folin Wu method. The final dilution is made to 10 cc.

With standard set at 20 and X = reading of the unknown for the 0.08 mg standard $\frac{2400}{X}$ = mg glucose per 100 cc of blood and for the 0.1 mg standard $\frac{3000}{X}$ = mg glucose per 100 cc of blood.

Comparison with the macro Folin Wu sugar method¹ on the same samples were as follows:

MG SUGAR PER 100 CC OF BLOOD

SAMPLE	MACRO	MICRO	DIFFERENCE
1	179.6	178	0.9 %
2	109.0	112.1	3.0 %
3	106.5	111.7	1.6+%
4	105.7	106.8	0.2+%
5		101.2	0.4+%

REFERENCE

¹Folin, O., and Wu, H. Jour Biol Chem, 1920, 31, 367

METHODS FOR MAKING A STABLE EMULSIFIED SYPHILITIC ANTIGEN*

BY FREDERICK PROESCHER, M D , ALBERT ARKUSH, A B , AND ALBERT KRUEGER, AGNEW, CALIFORNIA

ALCOHOLIC heart extracts, plain or fortified with cholesterol and acetone insoluble lipoids of normal tissue, are at present the antigens of choice for the syphilitic complement-fixation test

Ethyl alcohol 96 per cent (or absolute), methyl or propyl alcohol has been found so far to be the best extraction medium. In order to obtain a good antigen the wet or dried tissue should be extracted at least five to eight days or even longer at 38° C to secure the maximum of antigenic activity

For several years Dr Proescher has conducted a large number of experiments to find another extraction medium which shortens the extraction time and removes the maximum of the specific lipoids without removing the anti-complementary and hemolytic substances

The following fat solvents were used: chloroform, carbontetrachlorid, carbonbisulphide, pyridin and petrol ether. Chloroform gave the best results either with wet or dried tissue, almost equally as good was pyridin, but on account of its disagreeable odor this was discarded

The alcoholic extracts must be carefully diluted with saline solution in order to obtain a suitable antigen. The state of dispersion greatly influences the antigenic properties. Saline diluted alcoholic extracts are unstable. The advantage of a stable emulsified antigen is obvious

The original antigen was prepared from fresh beef hearts by direct extraction with chloroform. This antigen will be designated as "Chloroform Antigen I" and contains the neutral fats plus the specific lipoids (Lecithin, Phosphatides) and a natural amount of cholesterol (0.1 to 0.13 per cent). Since the neutral fats are without antigenic properties and make the final emulsification somewhat difficult, we have lately used dried beef heart and the neutral fats were completely removed by petrol ether,† previous to the chloroform extraction. This new antigen will be designated in the following as "Chloroform Antigen II", it does not contain cholesterol

PREPARATION OF "CHLOROFORM ANTIGEN I"

To 500 gm of fresh, preferably not chilled, finely ground beef heart add 250 cc chloroform‡ and agitate for twenty-four hours in the shaking ap

*From the Pathological Laboratory of Agnew State Hospital

Received for publication January 17 1927

†The average neutral fat content of 9 dried beef hearts was 11.1 per cent the lowest was 8.58 per cent the highest 14.45 per cent. The average chloroform soluble lipid content of 7 beef hearts extracted at room temperature for twenty-four hours previously extracted with ether was 3.08 per cent the lowest was 2.84 per cent the highest 3.23 per cent. The water content of 7 dried beef hearts was in the average 5.2 per cent the lowest 4.05 per cent the highest 5.95 per cent. The ether extract contained 0.15 to 0.2 per cent cholesterol

‡The chloroform should be acid free and preferably distilled before use

paratus at 37° C*. Decant the chloroform off by draining the bottle over a funnel. The chloroform lipid solution is then filtered repeatedly through a thick, dry filter paper until perfectly clear.

The filtrate, which is usually of a golden yellow color, is poured into a crystallizing dish and the chloroform evaporated with the aid of a fan at room temperature. The chloroform should be entirely evaporated by stirring the fatty material with a glass rod until the last traces of chloroform are driven off. Add to the lipid material, which is of a creamy consistency, about 50 gm washed and ignited sea sand and 10 c.c. redistilled 96 per cent alcohol. Mix thoroughly and transfer into a clean heavy walled, narrow mouthed bottle, and add 200 c.c. of normal saline solution containing 0.3 per cent carboic acid. The carboic acid saline solution should be slowly added with vigorous shaking. For proper emulsification the bottle should be agitated in a shaking apparatus for 24 hours. The resulting milky emulsion is finally filtered through a loose cotton plug to remove the sea sand and unemulsified lipoids.

We usually extract portions of 4 or 5 beef hearts separately at the same time since not every heart gives a satisfactory antigen. Experience has shown that if the chloroform soluble lipoids do not emulsify and only a slightly opalescent but not milky emulsion is obtained its antigenic properties are too weak to be used. About 40 per cent of the hearts will give a satisfactory antigen.

PREPARATION OF CHLOROFORM ANTIGEN II''

Fresh beef heart is finely ground in a meat grinder spread out in a thin layer on glass plates and dried† at 37° C in the incubator with the aid of a fan. The dried muscle is ground again in a mill until a fine powder is obtained. If not perfectly dry, it is spread out in a thin layer on glass plates and dried for several days at 37° C. The dried powder is stored in amber colored bottles and will keep for several months. Fifty to 100 gm of powdered beef muscle is filled in a paper extraction thimble and for three days extracted in the Soxhlet Extraction Apparatus with petrol ether. After a complete extraction the contents of the thimble are spread out on a glass plate and dried until the ether is completely evaporated. The ether removes besides the neutral fats and cholesterol, the fatty acids. The ether free heart muscle is filled in a narrow necked amber colored glass bottle and 100 to 200 c.c. redistilled chloroform added and agitated for twenty four hours at room temperature (or incubator 37° C) in the shaking apparatus. The chloroform extract is filtered through a thick filter paper and the clear chloroform solution poured in a crystallization dish the chloroform completely evaporated and the remaining lipoids emulsified in a 100 to 150 c.c. phenolized saline solution as described above.

*If a shaking apparatus is not available the extraction may be accomplished in the incubator with occasional shaking.

†For rapid drying the ground heart muscle (500 gm.) is mixed in a large beaker with 1000 c.c. acetone and let stand for an hour with occasional stirring. It is filtered through a Buchner funnel, spread on glass plates and dried at 37° C. The acetone acts as a dehydrating agent and removes some of the neutral fats without removing the specific lipoids. In this way large amounts of ground heart muscle can be dried in a short time without the aid of a fan.

If desired the lipoids may be kept in a vacuum desiccator over concentrated sulphuric acid or phosphoric anhydride. If protected from light and kept at ice box temperature they will keep indefinitely.

Lately we have evaporated the chloroform extract in large thick walled test tubes ($20 \times 2\frac{1}{2}$ cm) and after complete evaporation of the chloroform, the remaining lipoids are dried in a vacuum desiccator over phosphoric anhydride. After a week's drying, the end of the test tube is drawn out into a small tube and connected with the vacuum pump and evacuated as completely as possible and sealed while still in connection with the vacuum pump. The sealed tubes are stored in the refrigerator.

If an aliquot part of the original chloroform extract is evaporated, emulsified and titrated against a polytropic syphilitic serum, measured amounts of the chloroform extract may be evaporated in a large test tube, dried, sealed, and for use a definite amount of phenolized saline solution added.

In this way a standardized amount of lipoids can be kept on hand ready for immediate use.

PROPERTIES OF EXTRACTS

The antigenic, anticomplementary and hemolytic properties of the chloroform-soluble lipoids compare favorably with alcoholic heart extract fortified with cholesterol and the acetone insoluble lipoids of normal tissue.

An ideal extract should be highly antigenic and as little hemolytic and anticomplementary as possible. If used in doses of two to four antigenic units its anticomplementary and hemolytic properties should be at least ten times less than its antigenic properties. The antigenic activity of a good "Chloroform Antigen I" should be at least 0.05 cc if titrated against 0.1 cc of a mixture of several syphilitic sera using 2 or 3 units ofamboceptor and 3 or 4 units of complement. Very good antigens may protect in doses of 0.005 to 0.001 cc.

With the Method II we were able to obtain in 100 per cent of the hearts a suitable antigen of even higher potency than with the original method.

The following table gives the titer of 14 different antigens* titrated as above.

EXTRACT	AMOUNT OF EXTRACT GIVING COMPLETE INHIBITION C C
1	0.004
2	0.001
3	0.0001
4	0.0001
5	0.002
6	0.0001
7	0.001
8	0.0002
9	0.0001
10	0.001
11	0.006
12	0.001
13	0.0002
14	0.0006

*Chloroform soluble lipoids of 9 gr of dried beef heart emulsified in 15 cc saline.

All of the above antigens were neither anticomplementary nor hemolytic in doses of 1 c c of the undiluted antigen

Kolmer has called attention to the fact that a good antigen should be highly polytropic or, in other words that it should have affinity for the lipodophilic antibodies in the sera of all syphilitics. The chloroform antigen seems to be slightly more sensitive than the fortified alcoholic heart extract, if the ice box incubation is used. In 104 cases of paresis the blood Wassermann was positive in 97 per cent with the chloroform antigen and in 95 per cent with the cholesterolized alcoholic beef heart. In untreated cases of secondary syphilis results were positive in 98 per cent of the cases.

The advantage of the chloroform antigen is the great stability of its antigenic properties without becoming anticomplementary or hemolytic. We have so far never encountered an extract which would not keep at least for one year, while alcoholic heart extracts may become anticomplementary in a few weeks.

A NOTE ON THE MEASUREMENT OF BLOOD FOR CHEMICAL EXAMINATION*

By S. L. LEIBOFF, A. M. NEW YORK, N. Y.

WHILE we pay a great deal of attention to the refinement of technique in the chemical examination of blood we usually overlook one important factor which introduces a source of error and that is the accurate measurement of the blood.

The assumption that the pipettes at our disposal are quite accurate is an erroneous one. Thus when I tested a dozen Kolmer blood pipettes picked at random, for their accuracy, I found great variations in the amounts of blood delivered by the various pipettes. The blood was drawn into each pipette from a flask containing citrated sheep blood to the 5 c c mark, and weighed in stoppered weighing bottles, thus avoiding loss by evaporation. The blood was shaken well each time in order to avoid variations in the proportion of cells to plasma, and at no time was any sample of blood, after being withdrawn, returned to the flask since during the transfer the proportion of cells would diminish, the cells being more viscous than the plasma, more would stick to the sides of the pipette and weighing bottle, thus introducing a source of error. The largest variation in the weights of the blood delivered by the different pipettes was 4.6 per cent.

Another source of error is introduced by the inequality in the diameter of the tip in different pipettes, since the greater the diameter of the tip the faster the blood will flow, thereby leaving a greater amount of blood stuck to the walls of the pipette particularly since blood is a rather viscous fluid.

Still another error is introduced by the variations in the viscosity of the

blood in various diseases. The viscosity of a liquid is the resistance it offers to flowing or changing its shape, since in a liquid the size of the molecules relative to the space between them is so large that the molecules get into each other's way, thus limiting their freedom of movement. Accordingly, the higher the viscosity of the blood the more of it will stick to the sides of the pipette, thereby delivering a lesser amount. The great viscosity of the blood is due chiefly to the corpuscles, but also to the proteins by reason of their being hydrophilic colloids.

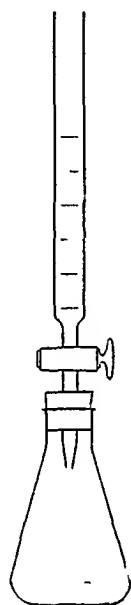


Fig 1

The simple device shown in the picture is recommended to overcome these difficulties. It is a short burette with a glass stopcock, about 15 mm in diameter. It can easily be standardized to a high degree of accuracy by placing into it 13.5585 gm of pure mercury for every cc of blood and marking the glass at the meniscus of the mercury, this being the weight of 1 cc of mercury at 15° C which is the usual laboratory temperature.

To measure out blood, the burette, with the stopcock closed, is filled with the desired amount of blood, taking care not to introduce any blood above the washed down with the amount of water required for the dilution of the blood mark. The stopcock is then opened and the blood clinging to the burette is

A PORTABLE THERMOELECTRIC APPARATUS FOR THE DETERMINATION OF SURFACE AND TISSUE TEMPERATURES*

By H C BAZETT, M.D., AND B MCGLOTHLIN, D. PHILADELPHIA, PA.

THERMOELECTRIC determinations of the temperature of human tissues were reported by Becquerel and Breschet (1835) and this method has been employed in numerous subsequent investigations. As shown by these authors (1839) many of the obvious difficulties attendant upon the use of mercury thermometers are eliminated and the errors common to both methods are reduced when thermocouples are employed. Benedict (1925) in discussing the temperatures of the surface of the skin has also pointed out the advantage of thermocouples of small dimensions capable of close contact as compared with the relatively large bulb of mercury instruments. To adapt the cumbersome laboratory equipment to clinical use and to maintain a high degree of accuracy this portable thermoelectric apparatus has been constructed.

In Fig 1 ($\lambda 1\frac{1}{2}$), it will be noted that the apparatus consists of two thermocouples (*S* and *N*), a constant temperature bath (*B*) and galvanometer (*G*). The surface thermocouple (*S*) is of the type suggested by Benedict (1925), employing, however, as thermoelements constantan and iron and wire of smaller diameter (0.32 mm). The constantan wire leads from the variable thermojunction (at *S*) to the constant junction within the bath (*B*), a length of about 130 cm, entering the bath by a lead (*d*) at this point an iron wire is soldered, forming the constant thermojunction, and this wire is continued as one of the galvanometer leads (*a*). The iron thermoelement from the variable junction leads directly to the galvanometer by lead (*e*). The wires are silk insulated, and to secure further insulation and rigidity they are enclosed in rubber tubing (inside diameter, 8.0 mm, thickness of wall 2.0 mm). At the exits from this larger tubing each wire is enclosed singly in tubing of thinner wall and of 5.0 mm external diameter. The total resistance of this thermocouple and its leads should be about 110 ohms and in the example described is 118 ohms.

A steel constantan thermocouple of needle type which is a modified form of the design of Lefevre (1898-1911) is employed for the determination of dermal and subcutaneous temperatures. Fig 2 shows the construction of this needle thermocouple. Steel tubing (*1*) as drawn for hypodermic needles 0.45 mm diameter, of 14.5 cm total length is supported for 8.7 cm of its length in a hard rubber cylinder (*2*) the needle is fixed at both ends of the cylinder by plaster of Paris leaving between these points of fixation an insulating air space. The steel tubing protrudes from the hard rubber support a length of 5.8 cm and is protected by a cylindrical cover of brass (*3*) 8.3 cm in length.

From the Department of Physiology, Medical School, University of Pennsylvania.
Received for publication February 3, 1927.

and of 1.4 cm external diameter, as the thickness of the brass wall is about 0.3 cm a considerable air space is obtained. This brass cover is continuous below with a hard rubber cone (4) of 1.1 cm length and support is thus given to the needle, which touches the hard rubber cone at the exit of the needle for a length of 1.0 mm. The hard rubber cylinder (2) has a screw thread (pitch usually 1 mm), the brass cover (3) has a detachable screw head (5) which fits the thread of the hard rubber cylinder (2). Thus the brass cover may be adjusted to give a variable length of needle beyond the tip of the hard rubber cone, and by reference to the number of turns of the cover (3), the distance exposed, and so the depth to which the thermojunction is buried

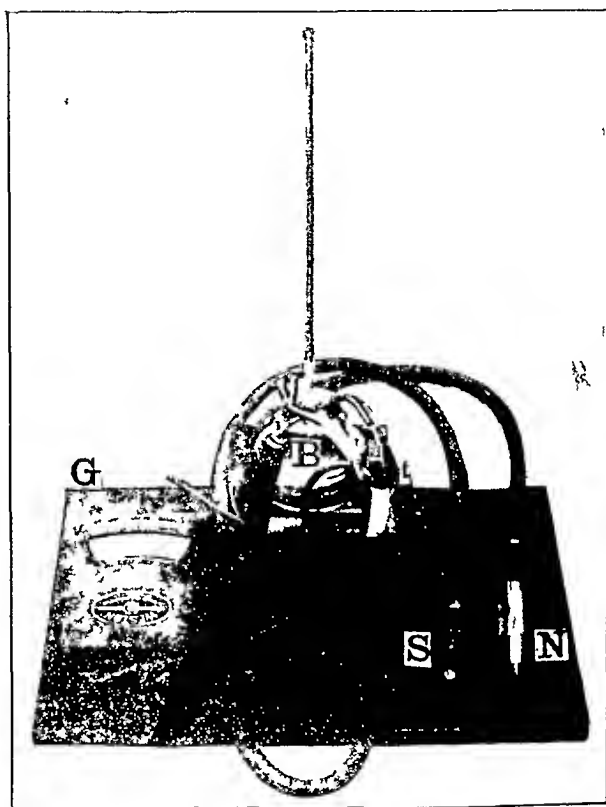


Fig 1—Portable thermoelectric apparatus (XI)—Description in text

in the tissue can be ascertained. At the upper end of the steel tubing, a lead of similar steel tubing (of 0.36 mm diameter) is soldered, and without further break, this leads to the galvanometer (Fig 1, G). Through the steel tubing in the needle proper insulated constantan wire (0.127 mm diameter) is threaded, and soldered to the steel at the beveled end of the tubing (Fig 2, J), this constantan wire is continued without break to the constant temperature thermojunction in the bath, which junction is made by soldering to the constantan steel tubing which in turn serves as the galvanometer lead. The wires are enclosed and supported in rubber tubing as previously described. In this case the leads were made up of two strands of steel tubing and suff

cient strands of constantan so as to reduce the total resistance to 12.9 ohms. The galvanometer leads of both surface and needle thermocouples are readily detached from the binding posts of the galvanometer to facilitate rapid substitution.

The constant temperature bath (Fig. 1 *B*) consists of a thermos flask of one pint capacity, containing paraffin oil, and sealed with cork. Through perforations in the cork the constant temperature thermocouple leads enter the bath and in addition the cork supports a thermometer, so adjusted that its bulb is at the level of the thermojunctions.

The galvanometer is of the double pivoted movable coil type (Weston model 440), of an internal resistance of 35 ohms, a period of 2.3 seconds, and a deflection indicating 2.2 microamps per division.

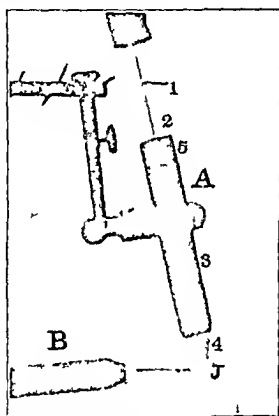


Fig. 2.—X-ray photograph of thermocouple needles (X₁). Description in text. Thermocouple A is inserted radially and B obliquely into the subject's arm. The dotted line indicates the margin of the skin.

As can be seen in Fig. 1 both the galvanometer and constant temperature bath are secured to a baseboard and spring clamps support the thermocouples when not in use.

The constant temperature bath is kept at room temperature. To determine the "temperature factor" or value in degrees of temperature per scale division the thermocouples should be standardized against a mercury thermometer in water, preferably at least once during each experimental day, and the standardizing temperatures, two or more should if possible be higher than that of the constant bath unless the environmental temperature should by chance be higher than that of the surface or tissue studied.

Due care should be observed to prevent rusting and the exposed portions must after using or standardizing be dried and coated with a pure vaseline. The mercury thermometers employed in this set are about 40.0 cm. in length and graduated from -10.0° to 50.0° C. in $\frac{1}{10}$.

The sensitivity of the set illustrated is between 0.6° and 0.8° C per scale division, insuring an accuracy of at least 0.2° C in the estimation of the thermocouple temperature. If the needle be inserted obliquely into the tissues to a length of 10 mm or more (as illustrated in Fig 2, needle B), the thermocouple causes only slight thermal disturbances in the tissues and the temperature so determined may be considered as that of the tissue. If on the other hand the needle be vertically inserted to a small depth (less than 5 mm, as needle A, Fig 2), the temperature gradients are modified by the presence of the needle and the temperatures calculated are considerably lower than those normally existing in the tissues. At room temperatures of 22° or 23° the calculated temperatures are approximately too low by 0.5° for a depth of 3 mm, 0.3° for 5 mm, and 0.2° for 7 mm. The actual depth of the thermal junction in obliquely inserted needles can be estimated from a consideration of the angle of insertion. Needle thermocouples have been described by Adrian and Watts (1923), and have been employed by Lewis and Love (1926). Needles of the type described by the former authors are less accurate than those here described, since complicating factors are introduced by the use of an ordinary hypodermic needle, which is nickel plated and, in addition, usually has a brass seating.

REFERENCES

- Adrian, E. D. and Watts, C. F. *Jour. Physiol.*, 1923, *lx*, 11.
 Becquerel et Bieschet. *Ann. de Sc. Nat.*, 2^e sér., 1835, *iii*, 257.
 Becquerel et Bieschet. *Arch. du museum d'Hist. Nat.*, 1839, *i*, 383.
 Bénédict, F. G. *Ergebn. d. Physiol.*, 1925, *xiv*, 594.
 Lefèvre, J. *Arch. de physiol.*, Jan., 1898, 34.
 Lefèvre, J. *Chaleur animale et bioénergétique*, Paris, 1911.
 Lewis, T. and Love, W. S. *Heart*, 1926, *xiii*, 27.

A COLORIMETRIC METHOD FOR THE DETERMINATION OF IRON AND HEMOGLOBIN IN THE BLOOD*

By MARTIN DUPRAY B.S., M.S., HUTCHINSON KANSAS

COLORIMETRIC BLOOD IRON METHOD

WONG¹ has described a colorimetric method for the determination of the iron in the blood. In his method the organic matter of the blood is destroyed by sulphuric acid and potassium chlorate and the iron estimated as ferric thioeyanate.

The destruction of the organic matter in Wong's method requires immediate attention throughout the process as the boiling of the acid must be interrupted several times to add fresh potassium chlorate and this addition must be made with some care with the tube inclined to avoid spattering or even explosion.

I have found that perchloric acid is much superior to potassium chlorate in this digestion. The proper amount of perchloric acid may be added to the sulphuric acid to bring about complete digestion in ten or twelve minutes of boiling and with rare exceptions enough of the perchloric acid persists throughout this boiling to complete the digestion without further additions. The oxidation, while rapid is not violent. A little nitric acid included in the digestion mixture materially hastens the early stage of the digestion and shortens the total time a little. With the digestion mixture to be described, digestion is complete in from seven to ten minutes with very little attention required.

Wong uses a standard ferric iron solution prepared by oxidizing a ferrous ammonium sulphate solution to the ferric state with potassium permanganate. When this solution stands some time a brown sediment probably manganese dioxide appears. In the original oxidation manganese is reduced from the heptavalent to the divalent state and remains in solution as manganous sulphate. Its precipitation as the dioxide indicates a reversion to the quadrivalent state at the expense of the iron part of which is reduced to the ferrous state. This necessitates frequent additions of small amounts of permanganate to maintain a pink color indicating complete oxidation before using the standard, which in time dilutes the standard. Also the precipitate of manganese carries down traces of iron with it. I once found three milligrams of iron in the sediment formed in one liter of standard solution prepared by Wong's method some two months after preparation. This introduced an error of three per cent in the standard.

A more accurate standard can be prepared by using metallic iron as the starting point. Finely drawn iron wire of known purity is readily obtained

From the Dupray Laboratory, Hutchinson, Kansas.
Received for publication December 1, 1916

able from the supply houses, with the true iron content stamped on the packages. When this is dissolved in a moderate excess of *hot nitric acid* the resulting solution is ferric iron and remains so, and the above errors are avoided. (The use of a cold dilute nitric acid, or of sulphuric acid would yield a ferrous iron solution.)

In Wong's method the ferric thiocyanate color fades rather rapidly, probably from reduction by sulphuric acid. Also the tests fade less uniformly than the standards, probably due to varying amounts of chlorine in the digests. This makes necessary the addition of the thiocyanate to the test and standard simultaneously and reading of the colors at once. Even then, some errors, due to fading, are unavoidable. A little nitric acid added before developing the color prevents this fading. By using this procedure, standards and tests four hours old show no fading when compared with a freshly prepared standard.

These facts have been incorporated into the following method.

REAGENTS REQUIRED

1 *Digestion Mixture* Twenty-five c.c. of distilled water are placed in a small flask and 50 c.c. of iron-free sulphuric acid, specific gravity 1.84, are added slowly while stirring. The solution is cooled, after which are added 15 c.c. of 60 per cent perchloric acid, and 10 c.c. of iron-free nitric acid, specific gravity 1.42, and the solution mixed and transferred to a glass stoppered bottle.

2 *Potassium Thiocyanate Solution* The same as used by Wong, approximately 3 N, or 29.2 gm. of potassium thiocyanate per 100 c.c. of solution.

3 *Standard Ferric Iron Solution* A portion of finely drawn iron wire of known iron content is thoroughly cleaned with fine emery cloth or paper, until certain of the absence of rust and dirt. A sufficient quantity to give 100 mg. of iron is accurately weighed. For example, if the wire contains 99.8 per cent of iron, 100.2 mg. of the wire is weighed out. Ten c.c. of iron-free nitric acid, specific gravity 1.42, and about 40 c.c. of distilled water are placed in a small flask and brought to a boil. The wire is rolled into a loose coil and dropped into the boiling acid, together with any trimmings used in making the exact weight. Solution is complete in a few moments. The solution is boiled for about one minute after solution appears complete, then cooled. It is then transferred without loss to a 1000 c.c. volumetric flask, the small flask rinsed with several portions of distilled water and the rinsings added to the solution in the volumetric flask. Finally the solution is made up to 1000 c.c. with distilled water and mixed. Each cubic centimeter contains 0.1 mg. of ferric iron. The solution is permanent.

METHOD

One c.c. of well mixed oxalated blood is transferred to a test tube containing exactly 4 c.c. of distilled water, using an Ostwald-Folin pipette, preferably one graduated to deliver between marks. The diluted specimen is mixed and allowed to lake, after which it is mixed again, and 1 c.c. of the

diluted sample placed in a large pyrex test tube, 25 mm by 200 mm, with a graduation at 25 cc. One cc of the digestion mixture and two or three glass beads* are now added, and the tube placed in a holder over a micro burner. The contents are boiled gently until digestion is complete, which usually requires from seven to ten minutes. Most of the action takes place after the water is boiled off and white fumes appear. Very rarely a blood is encountered, usually one high in fats, which will not completely digest. If it is obvious, after twelve minutes or so of boiling, that digestion will not go to completion, remove the flame and allow the tube to cool slightly. Then add one or two drops of 60 per cent perchloric acid, and resume the digestion. A very little more boiling will then suffice. When digestion is complete the fire is removed and the tube cooled. The liquid will not usually be entirely colorless, when hot a more or less yellow color persisting due to chlorine and nitrous fumes. This color fades on cooling leaving a colorless liquid when cold. The tube may be allowed to cool to room temperature before adding water.

When cold, two or three drops of strong nitric acid are added to insure an excess, as the nitric acid in the digestion mixture may be all boiled out. The volume is then brought to about 15 cc with distilled water, and 5 cc of the thiocyanate solution are added after which the volume is carefully brought to 25 cc with distilled water, and the solution mixed. It is then ready to compare in the colorimeter with the standard.

The standard is prepared by placing 1 cc of the standard ferric iron solution (containing 0.1 mg of ferric iron) and 1 cc of the digestion acid mixture in a similar large test tube marked at 25 cc. Distilled water is added to about 15 cc. 5 cc of the thiocyanate solution are added, and the volume brought carefully to 25 cc with distilled water and mixed. There is sufficient nitric acid in the digestion mixture that no more need be added. Check the colorimeter cups against each other with the standard color solution. Then set the standard at 20 mm.

Calculation

$$\frac{\text{Reading of standard in mm}}{\text{Reading of unknown in mm}} \times 10 = \text{Milligrams of iron per 100 cc of blood}$$

Using 0.335 as the percentage of iron in hemoglobin

$$\frac{\text{Milligrams of iron per 100 cc of blood}}{3.35} = \text{grams of hemoglobin per 100 cc of blood}$$

The standard may be varied in strength so as to make the calculation direct in per cent of hemoglobin as related to some normal standard. For example, taking Haden's⁴ figure of 15.6 gm of hemoglobin per 100 cc of blood as normal, and 0.335 as the per cent of iron in hemoglobin the iron content of the blood at this normal becomes 52.26 mg of iron per 100 cc of blood. If then the standard ferric iron solution is made to contain 104.5 mg of iron per

Folin and Wu pointed out that the antibumping properties of glass beads and quartz pebbles came from the presence of air in the fine pores. Following up this idea it was found that one could obtain at a department store small glass beads known as satin beads which derive their satiny or frosted appearance from the presence of numerous small bubbles and striae in the glass. These beads have proved unusually efficient in preventing bumping.

liter instead of 100 mg, and 1 c.c. be used in the standard color tube, the computation becomes,

$$\frac{\text{Reading of standard in mm}}{\text{Reading of unknown in mm}} \times 100 = \text{hemoglobin in per cent of Haden's normal}$$

SUMMARY

A method for the quantitative colorimetric estimation of the non, and from it the hemoglobin of the blood is described. It is essentially a modification of Wong's method, but is somewhat less tedious, and avoids some of the errors of Wong's method.

REFERENCES

- ¹Wong, San Yin Jour Biol Chem, 1923, lv, 421
- ²Fohn, O, and Wu, H Jour Biol Chem, 1919, xxxviii, 81
- ³References cited by Wong ¹
- ⁴Haden, Russell L Jour Am. Med Assn, 1922, lxxv, 1496

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. MILDUFF, M.D., ABSTRACT EDITOR

PEPTIC ULCER Etiology and Pathology of Peptic Ulcer Levine S. Am. Jour. Med. Sc. July, 1920 clxxx No 1 p 22

From a survey of available information concerning peptic ulcer Levine thus summarizes the present status of knowledge of the condition.

Peptic ulcer starts as an erosion.

Erosions may be produced by mechanical, chemical, physical causes, circulatory and nervous disturbances, infection and all other phenomena.

Erosions in the region of the Mucosa are not healed readily because of its peculiar structure.

Erosions are converted into peptic ulcers by the destructive action of the acid gastric juice.

The pyloric canal and first portion of the duodenum because the reason they offer to the acid chyme are particularly liable to be involved in ulcerative process.

HEMORRHAGE Hemorrhages, Their Significance and Methods of Treatment, Mills, C. A. Am. Jour. Med. Sc., July 1920 clxxx No 1 p 1

In a scholarly review of hemorrhage which will repay reading in the original Mills recalls the efficacy of anaphylactic reaction as a means of treatment in hemophilia. The patient is sensitized by the subcutaneous injection of 4 cc of sheep erythrocytes and a reaction provoked even to ten days later by the intravenous injection of a few drops. If a single reaction does not bring the bleeding to a normal the reaction may be repeated at weekly intervals.

The injection of the mother's whole blood into the mother's subcutaneous tissue is an efficient method of treatment in hemorrhage of the newborn.

Mills looks forward to the discovery of the mechanism by which hemorrhage stimulates resistance to disease and to the introduction of blood plasma as a therapeutic measure.

PERNICIOUS ANEMIA Etiology and Treatment of Pernicious Anemia, Barker L. F. Jour. Am. Med. Ass. July 10 1920 lxxxv

Granting that it is literally true that neither the cause nor an effective treatment for this disease is as yet known, Barker summarizes the advances and additions made in the knowledge of the disease in recent years as follows:

1. Though the causes of pernicious anemia are not yet fully known clues to their nature are being obtained.

2. In any consideration of etiology the attention should be paid to the peculiarities of incidence and distribution of the disease to the fact that it is predominantly a malady of middle and later life to the characteristic features of the blood picture and their relations to blood destruction within macrocytes and to blood regeneration of embryonal type to the associated disturbances of the digestive nervous and endocrine system to certain peculiar marks in the bodily conformation to the occurrence of spontaneous and of therapeutically inducible remissions of variable duration and to the inevitability in the present state of knowledge of a fatal termination.

3. Of the many conceptions of etiology that have been advanced the evidence at present favors hereditary (genotypic) predisposition as the main factor and various influences in the external conditions (especially poisons derived from bacteria, fungi or animal parasites in the digestive tract, a defective or relaxing or provocative factors).

4 Parallel with the growth of hypotheses of etiology, conceptions of pathogenesis are being extended, the causes of the disturbances of equilibrium between blood destruction and blood regeneration (and their antecedents) are becoming clearer, the anemia is recognized as only one part of a comprehensive disease entity in which the digestive system, the nervous system and the endocrine system are also involved, and investigators are now striving to establish correlatives among the various phenomena observable and to find the precise place in the malady as a whole that should be assigned to each integral part

5 Treatment of the disease, though not curative, is rewarding Through rest, the administration of dilute hydrochloric acid and of arsenic, injections of blood and other measures, the patient's condition can often be greatly ameliorated, and in many instances remissions of variable duration may be induced Early recognition of achyha, paresthesias, glossitis, and of megalocytosis (before anemia develops) may permit prompt treatment that will tend to keep the malady latent Intermarriage of members of families in which the disease is known to occur should be discouraged

The paper contains a large amount of data succinctly expressed and should be read in the original

PERNICIOUS ANEMIA Blood Changes in Rabbits Resembling Those in Pernicious Anemia Accompanying B Welchii Infections, Reed, G B, Orr, H J, and Burleigh, C H Can Med Assn Jour, May, 1926, xvi, No 5, p 525

It has been shown that a highly virulent strain of B welchii may produce chronic or acute infection in rabbits depending upon the age of the culture used

Rabbits suffering from acute or chronic infection with B welchii develop a profound anemia characterized by a decrease in red cell numbers without a corresponding decrease in hemoglobin and by conspicuous anisocytosis

Quantitative examination of the degree of anisocytosis shows that it resembles that of pernicious anemia in man

These results are presented in the form of a progress report, other data are in process of publication elsewhere concerning the action of B welchii toxin on red blood cells both in vivo and in vitro and on other tissues

URIC ACID Uric Acid and Creatinine in the Urine of Infants, Roughitch, O S Am Jour Dis Child, April, 1926, xxvi, No 4, p 505

The uric acid excretion per twenty four hours was measured in a series of twenty two male infants The diet of the infants being purine free, the amounts of uric acid found are regarded as of endogenous origin In contrast with most of the hitherto published measurements of uric acid in infants' urine, a closely constant daily excretion was found for the individual, and a fairly constant value per unit of body weight for the group of infants, the range being from 14 to 25 mg of uric acid per kilogram of body weight

Except in the case of an infant with active rickets whose uric acid excretion was high, 36 and 34 mg per kilogram of body weight, no relationship of nutritional state to uric acid excretion was apparent Uric acid excretion was also apparently independent of the age of the infant

It is suggested that the relatively high level of excretion of endogenous uric acid by infants may be explained by their high protein intake, using the evidence obtained by Folin and his coworkers that a high intake of protein retards the destruction and accelerates the excretion of uric acid It must be admitted, however, that in this series the relationship of urine nitrogen to uric acid was not close, uric acid nitrogen ranging from 1.25 to 3.5 per cent of the total nitrogen

Measurements of urine creatinine in the same group of infants support the conception of a close constancy of creatinine excretion in terms of the protoplasmic mass of the body The range found was from 10 to 15 mg per kilogram of body weight The highest values were obtained in malnourished infants, probably because of their relatively larger mass of active tissue

No relationship of creatinine excretion to differences in sleeping time or of muscular

tonus was discernible. In the case of an infant having severe generalized convulsions, however, a creatinine excretion three to four times higher than normal was observed. Another infant with slight localized convulsions was found to excrete an approximately normal quantity of creatinine.

MERCUROCHROME The Present Status of Mercurochrome—220 Soluble Davis, H. B. *Am Jour Med Sc*, September, 1926, clxvi, No. 6, p. 340

There have been so many conflicting reports concerning this substance, many so biased or lacking in controls as to be difficult to evaluate, that Davis reviews the experimental work which has been done.

The paper first considers the favorable and then the unfavorable evidence, then the intraperitoneal use, the drug as a skin disinfectant, effect in edema, use in wounds, its precipitation by local anesthetics, and the reaction of the body to mercurochrome intravenously.

Seventy-six references are thus abstracted.

Davis concludes that there is experimental evidence of the value of mercurochrome—220 soluble intravenously in the treatment of septicemia and other infections. Other equally convincing experimental results point to the fact that it is not bactericidal in blood, and that its use is not unattended by danger. Many clinical reports show miraculous cures, others have no benefit, and in some it has probably hastened death. Therefore, treatment with mercurochrome must still be considered in the experimental stage. Because of its dangers it should not be used indiscriminately and should be reserved for desperate cases.

Mercurochrome is dangerous intraperitoneally because of the local irritant action and because of the often very severe general reaction.

If used in wounds, sinuses, or serous cavities its dose should be limited to 5 mg. per kilogram of body weight, as it is easily absorbed and if too much is used it may lead to severe reaction or stomatitis.

The alcohol acetone aqueous solution of mercurochrome recommended by Scott and Hill is a very satisfactory preoperative skin antiseptic. It should not be injected into the nose, urinary bladder, vagina, and so forth, however, along with a local anesthetic, as this will give a precipitate.

ANEMIA The Relation of Anemia Primary and Secondary to Vitamin A Deficiency Koessler K. G. Maurer S. and Laughlin, R. *Jour Am Med Assn*, August 14, 1926, lxxvii, 476

The opinion that the toxemia responsible for the symptoms of pernicious anemia is of intestinal origin is chiefly based upon the prominence of the gastrointestinal symptoms of the disease.

The authors believe that toxic substances or toxins are present in the intestinal tract in many instances but are not absorbed or are destroyed before absorption from the intestinal tract.

The problem seems to center around the query: "What are the conditions under which the normal viability and impermeability of the intestinal wall is lost?" Their belief is that a long standing deficiency of vitamin A may be responsible.

They regard pernicious anemia as, at least in certain cases, an intoxication through bacterial poisons formed sometimes by the colon bacillus at others by the streptococcus and Welch bacillus.

They conclude, from their experiments, that

1. Blood regeneration cannot take place without the presence of vitamin A.
2. The addition of vitamin A to the diet of animals, long depleted in their vitamin A reserve, brings about rapid formation of new blood cells.
3. The rate and intensity of the blood regeneration is a function of the quantity of vitamin A added.
4. A condition similar to human pernicious anemia has been produced in experimental animals.

5 A definite relationship exists between a state of chronic vitamin deficiency and certain anemias

6 The routine use of a rationally balanced diet which has proved itself thus far of decided value in the blood regeneration of patients suffering from severe anemias, aplastic as well as erythroid, is the most promising procedure in the treatment of certain anemias, especially pernicious anemia

SPIROCHETA PALLIDA Are There Immunologic Strains of *Spirocheta Pallida*? Kolmer, J A, Weiss, D, and Richter, C Jour Infect Dis, April, 1926, *xxviii*, No 4, p 318

An attempt to demonstrate by cross agglutination and complement fixation tests the existence of immunologically distinct strains of *S pallida*

No evidence of the existence of such was obtained in the study of six strains

On the contrary the results may lend some confirmation to the view that in so far as experimental syphilis of the rabbit is concerned, the localization of *Spirocheta pallida* and the subsequent course of the disease are largely influenced by the virulence of the organism and method of inoculation as well as by the susceptibility of the host and the efficiency of its defensive reaction Probably the same or similar factors are operative in syphilis of human beings without involving the question of strain specificity or "selective tissue affinity" or the infecting spirochetes

CARCINOMA The Mechanism of Cancer Metastasis, Burrows, M T Arch Int Med, April, 1926, *xxvii*, 453

Metastases in cancer are not the result of a simple migration of cancer cells from the cancer to distant organs Metastases are primarily the result of the spread of a liquid substance from the main tumor mass This substance spreads over surfaces It is liberated through a digestion of cells in the center of the mass of cancerous tissue This digestion is not an autolysis resulting from the absence of oxygen, but the result of an excess of the growth stimulating substance, a product of the cell's oxidation The fluid is rich in growth stimulating substance This fluid stimulates not only the cancer cells to grow but also the normal cells The cancer cells already adapted to it respond more quickly In their growth they then remove the nutrition and necessary substances from the other cells and destroy them

This type of reaction may not always occur As is well known the normal tissue may undergo malignant transformation Such has been seen frequently in transplanted cancers of animals These transformations are the result of a sufficiently long action of this fluid.

WASSERMANN Wassermann Reaction in Rabbit Syphilis, Wakerlin, G E, and Carroll, P H Jour Infect Dis, April, 1926, *xxviii*, No 4, p 327

The Wassermann reaction in rabbit syphilis is an index of tissue spirochete interaction and is not a criterion of the presence or absence of the spirochete

The Wassermann reaction is consistently positive in about 99 per cent of the cases of active rabbit syphilis following intratesticular inoculation

The appearance of the positive Wassermann reaction in rabbits affected with syphilis may be completely suppressed by the institution of adequate treatment in the clinically active but prepositive Wassermann stage

ANAEROBIC INFECTION Anaerobic Infection The Process, Dayton, N A Boston Med and Surg Jour, June 3, 1926, *xciv*, No 22, p 1032

The process of infection by gas bacilli depends on conditions which depress the vitality of the tissues and produce a rupture in the normal defenses This interference may be accomplished by, (a) the introduction of toxin or substances such as calcium salts, the coloids or sterile distilled water, (b) any factor withdrawing the defenses of the blood from the site of injury, such as continued cold, shock, and mechanical or surgical interference with the circulation of the part

The toxins are two in number and have a local and a hemolytic action, one extending the tissue injury and the other attacking the red cells by lysis. The toxins have a selective action on the suprarenal glands resulting in a complete paralysis of these organs.

The process in infections arising from the gastrointestinal tract differs decidedly from that of a tissue injury. The bacilli have difficulty in establishing themselves and seek an already existing pathologic lesion as a locus of attack or as a means of entering the blood stream. Anaerobic infections have been associated with gastric and typhoid ulcers, gall bladder disease, abscess and carcinoma of the liver and inflammations of the appendix.

The long continued presence of the gas bacilli in the blood stream without the terminal symptoms suggests a chronic infection. This is significant as the blood picture of chronic gas bacillus infection bears a close resemblance to that of pernicious anemia.

URINE SEDIMENT The Number of Formed Elements in the Urinary Sediment of Normal Individuals. *Addis T Jour Clin Invest* June 1926 11 No 5 p 409

The rate of excretion of casts, red blood cells and white blood and epithelial cells was determined in seventy four medical students under conditions favorable to the preservation of these urinary constituents. The following results were obtained:

RATE OF EXCRETION PER TWELVE HOUR PERIOD

	AVERAGE	LOWEST	HIGHEST
Casts	1040	0	4210
Red blood cells	65750	0	425000
White blood and epithelial cells	322500	32400	1835000

SYPHILIS Malaria in the Treatment of General Paralysis. Report of Cases. *Ridgeway E F L and Green E M Atlantic Med Jour May 1926 211 544*

Thirty four patients suffering from general paralysis who were treated by inoculation with blood from one having malaria of the tertian type.

Twenty six of these patients recovered from the inoculated disease. 7 died, and 1 failed to become infected. Of the 26 patients 5 were not benefited by the treatment. 9 were improved in greater or lesser degree, and 12 exhibited complete remissions. Seventeen of the number have been paroled from the hospital and 10 are still at home, many of them having reengaged in their former occupations.

The results obtained in the series of case prove that there may be no correspondence between the mental improvement and the changes in the physical signs of the disease nor in the serologic picture. Several of those manifesting complete remissions exhibited no favorable modifications in these respects. In only a small proportion of the cases did the Wassermann tests of the blood and the spinal fluid show changes which could be attributed to the malarial process. While physical signs were favorably modified in a number of instances in no case did they all show a return to the normal.

BLOOD Normal and Pathologic Fragmentation of Red Blood Cells. The Phagocytosis of These Fragments by Desquamated Endothelial Cells of the Blood Stream. The Correlation of the Peroxidase Reaction with Phagocytosis in Mononuclear Cells. *Doan C A and Sabin F R Jour Exp Med* June 1926 411 No 6 p 839

There is constantly some breaking down of the red cells in the circulation by fragmentation.

The fragments of red cells, as well as whole red cells are phagocytized and destroyed by clasmatocytes or endothelial phagocytes.

When there is an increase in fragmentation in abnormal or pathologic states desquamated endothelial cells of the blood stream as well as the clasmatocytes of the tissues increase proportionately and take in these fragments. These cells are to be distinguished from cosinophilic leucocytes by the nature of their granules by the type of motility of the cells and by a negative peroxidase test.

The desquamated endothelial cells, clasmatocytes, in the circulating blood are positive to the peroxidase test only when they have taken in positive material

The monocytes show marked variations of the oxidase reaction in different species and to different techniques. With the Sato and Sekiya technic most monocytes of human blood are positive, while most of them in rabbit blood are negative, but both positive and negative reactions are found in both human and rabbit blood

URINARY SEDIMENTS The Effect of Some Physiologic Variables on the Number of Casts, Red Blood Cells and White Blood Cells and Epithelial Cells in the Urine of Normal Individuals, Addis, T Jour Clin Invest, June, 1926, 11, No 5, p 417

Quantitative determinations of the number of formed elements in the urine of normal individuals failed to show that either bodily movements of various types or the ingestion of a large amount of protein in the form of meat had any statistically significant effect

ANTHRAX The Immunization of Sheep by Means of Anthrax Bacilli Attenuated with Sodium Chloride, Schilling, S J Jour Infect Dis, June, 1926, XXXVIII, No 6, p 499

A single injection of a sodium chloride attenuated culture of B anthracis protected sheep against subsequent infection with a virulent culture, the control animals died in fifty four hours of typical anthrax. The immunity conferred by the vaccine was general. Vaccination and subsequent inoculation with virulent cultures were performed subcutaneously but in widely separated sites of administration

Virulent anthrax bacilli may be found at foci of previous infection for some time following the apparent recovery of the infected animal

The reduced pathogenicity as well as the antigenic properties of the sodium chloride attenuated culture of B anthracis appeared to remain fairly constant during cultivation on artificial medium

ANTHRAX The Attenuation of B Anthracis by Means of Sodium Chloride and Other Chemicals, Schilling, S J Jour Infect Dis, April, 1926, XXXVIII, No 4, p 341

It was found that sulphuric acid and copper sulphate hydrolyze agar when added to this medium, even in such dilute concentrations as would not be expected to inhibit growth of B anthracis

The addition of 4.5 per cent sodium chloride and the addition of 1 per cent potassium ferrocyanide to standard agar appears to represent about the maximum concentration of these chemicals which may be used without completely inhibiting the growth of the anthrax bacillus. The growth inhibiting concentration of sodium hydroxide is about 0.15 per cent

An increased tolerance to sodium chloride, potassium ferrocyanide and sodium hydroxide could be noticed in successive transfers of the anthrax bacillus, as judged by the production of a more luxuriant growth

After growing the anthrax bacillus for seven weeks on agar containing 1 per cent potassium ferrocyanide, and for the same length of time on agar containing 0.15 per cent sodium hydroxide and testing the culture by inoculating guinea pigs, no decrease in virulence of the organism could be detected

After growing the anthrax bacillus for six weeks on agar containing 5 per cent sodium chloride, marked attenuation of the anthrax bacillus was demonstrated by guinea pig and rabbit inoculation

Attempts to immunize guinea pigs with the NaCl attenuated culture failed. Presumably this was because sufficient intervals of time were not permitted to elapse between injections

It was found that rabbits could be successfully and safely immunized by the use of the culture attenuated by growing on 5 per cent sodium chloride agar, so that they withstood the injection of virulent cultures of the anthrax bacillus in quantities which are regularly fatal to normal animals

REVIEWS

Books for Review should be sent to Dr Warren T Vaghan Medical Arts Building
Richmond, Va

*Obstetrics**

ONE volume of the Oxford Medical Handbook Series The purpose of this series is to deal shortly with the fundamental principles which underlie various subjects and to illustrate these by their proper application in general practice This volume gives a brief and concise presentation of the subject, including the physiology and pathology of reproduction, the management of pregnancy of normal labor and of abnormal labor

Laboratory Outlines in Bacteriology and Immunology†

A LABORATORY guide for the use of instructors in outlining their courses in elementary bacteriology and one that may well be used by the student as a manual which must be supplemented by personal instruction The work as outlined provides for instruction over a period of one year but is so arranged that the material may be utilized for courses of much shorter duration In addition to the usual elementary bacteriologic laboratory technic and study of representative microorganisms, a section is devoted to serologic technic including vaccine preparation, hemolysis bacteriolysis complement fixation flocculation tests, the coloidal gold test, phagocytosis, toxins and antitoxins and hypersensitivity

It should find a wide usefulness in its field.

Birth Control‡

A SYMPOSIUM by various authors on facts relative to birth control which should be in the hands of every physician This is not a disquisition on the relative value of contraceptives but is a clear exposition of the problem as it stands today and a consistent argument for the adoption of some satisfactory method of birth control The authors take no definite stand as to what methods shall be preferable

The human race has been here at least 400,000 years In the thousands of generations the population of the globe had increased up to 850,000,000 in 1830 Within the intervening century this population has been doubled It is estimated that one hundred years from now there will be three and one half times the present population or about five billions. It has

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto

Obstetrics By John S Fairbairn M.A. B.M. B.Ch (Oxon) F.R.C.P. (Lond) F.R.C.S. (Eng) Cloth Illustrated Pp 221 Price \$1.75 Humphrey Milford Oxford University Press

Laboratory Outlines in Bacteriology and Immunology By John F Norton Ph.D. and I S Falk Ph.D. Cloth Pp 114 The University of Chicago Press Chicago Ill. 19 6.

Birth Control—Facts and Responsibilities—A Symposium Dealing with this Important Subject from a Number of Angles Edited by Adolf Meyer M.D. Cloth Pp 167 Price \$3.00 The Williams and Wilkins Company 19 5

been estimated that five billion people are the most that can be fed if every tillable acre in the world is tilled as well as we know how to do it

Those, whose religion opposes birth control, insist that we are commanded in the Bible to increase and multiply and replenish the earth. They seem to forget that this command has already been obeyed in full, and that the command was given as an emergency procedure after a terrible flood in which all but eight people had been drowned. Since that time the population of the globe has been multiplied two hundred and fifty million times.

We hear much of the declining birth rate. This sounds bad until we realize that concomitant with this the death rate is shrinking still faster due to improved methods of preventive and curative medicines.

The autogonist often replies that by the time we become over populated new methods of synthetic food preparation will relieve the situation. They do not realize that not a single plant of dietary importance has been brought into cultivation within historical times. Prehistoric man discovered them all and now that there are no unexplored regions the probability of new boons like corn and potatoes is small. The limits of production will be expanded, the ravages of insects and fungi reduced but neither genetics nor chemistry can be expected to furnish a final solution to the old malthusian proposition.

War is but a temporary check to population. This requires no argument in substantiation, only census figures. The same is true of pestilence and famine.

Sentimentalists insist that all are born equal. The farmer knows that his animals are not born equal and he saves the well bred for breeding purposes and destroys the others. Human mating today is controlled chiefly by propinquity, religion, race, social position and personal attraction. Eugenics is not a dead science nor is it purely theoretical. Some day it will again come to the fore in a more practical aspect.

*Obesity*¹

DR LEONARD WILLIAMS has written a very readable treatise against fat people. It is for lay consumption primarily. The unlovely condition called corpulence has been divided into three stages known respectively as the enviable, the comical, and the pitiable. He does a place to the enviable obese. No degree of obesity is enviable.

The author draws widely on known facts of nutrition and metabolism and endocrinology and intersperses with them some of his own theories.

He has far less patience with the obese man than with the obese woman. Obesity in the male is, in his opinion, nearly always due to gourmandism and is therefore disgusting. Obesity when it occurs in the female is, in his opinion, usually endocrine in origin and therefore excusable. The fatter sex may, therefore, read the volume without gross injury to personal pride. Indeed, the author rather retraces his steps after his flagellation of the male for he lauds the slight corpulence of motherhood while decrying the boyish figure of the cocotte. In the former small accumulations of fat are physiologic in preparation for the increased metabolic drain of pregnancy and the maternally inclined woman, who looks upon sexual congress as a means to an end, is allowed to put on a little excess adipose tissue without criticism while the latter in whom the sexual act is an end in itself is severely criticised for keeping her figure slim so that it may be attractive to the male. This volume should be a source of gratification to the German hausfrau.

The physician may read this volume for relaxation and in it will find many good convincing arguments for use in his consultation work.

¹Obesity and Old Age By Leonard Williams M.D. Author of Minor Maladies and Middle Age Cloth Humphrey Milford Oxford University Press

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO JUNE 1927

No 9

Editor in Chief WARREN T VAUGHAN, M D

Richmond Va

ASSOCIATE EDITORS

DENNIS E JACKSON M D	CINCINNATI
PAUL G WOOLLEY M D	LOS ANGELES
J J R. MACLEOD M B	TORONTO
W C MCCARTY M D	ROCHESTER MINN
GERALD B WEBB M D	COLORADO SPRINGS
VICTOR C MEYER PH D	IOWA CITY
RUSSELL L HADEN M D	ROSEDALE ILL
JOHN A KOLMER M D	PHILADELPHIA
ROBERT A KILDUFFE M D	ATLANTIC CITY N J
A PARKER HITCHENS M D	MANILA P I
DEAN LEWIS M D	BALTIMORE
GEORGE R HEPRMAN M D	NEW ORLEANS
HAROLD D CAYLOR M D	ROCHESTER MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis Mo as Second Class Matter

EDITORIALS

The Etiology of Granuloma Inguinale

GRANULOMA inguinale is described by Fox¹ as a chronic infectious ulcerative process usually but not necessarily involving the genitalia or neighboring parts, showing little or no tendency to spontaneous healing, and yielding to treatment with tartar emetic (antimony and potassium tartrate).

Originally recognized and first described as 'serpigenous ulceration of the genitals' by McLeod in India in 1882, it was first isolated as a clinical entity by Coucyers and Daniels² in 1896 and since that time has been described under various names in various localities.

While long regarded as a tropical disease, the reports of numerous investigators have shown a rather wide geographical distribution and it is of particular interest to note that recent reports indicate that this disease is endemic in various parts of the North and South and is much more prevalent in the United States than is generally supposed.

There has been, and still is, much discussion as to the etiology of this infection. By many observers for a long time the inclusion bodies first described by Donovan³ in 1905 and now generally known as "Donovan bodies" have been regarded as the etiologic agent.

The occurrence of these bodies is an accepted fact in the lesions of this disease and the present controversy concerns their nature rather than their relation to the lesions.

There is some reason to question the identity of these bodies with the genus *Leishmania* and various workers have presented more or less convincing evidence that the bodies seen in this disease are not protozoal forms but bacteria of the *B. mucosus capsulatus* group.

On the one hand there is, as yet, no convincing evidence of the cultivation of *Leishmania*, and no evidence in any other bacterial disease of the specific action of a synthetic drug, and, on the other, some very suggestive reports are available of inoculation experiments with cultures isolated from granuloma lesions.

Lynch,⁴ for example, has cultured an organism morphologically similar in smear and culture which he regards as of etiologic importance and which he further believes distinct from the Donovan bodies of whose etiologic relationship to granuloma inguinale he is not altogether convinced. Campbell⁵ suggests that the Donovan bodies may be secondary invaders, while not committing himself as to the etiologic importance of the encapsulated organism isolated from his cases, and numerous similar reports can be found in the literature.

It may be granted that encapsulated and more or less pleomorphic bacteria can be cultured from a large percentage of cases, that they not infrequently appear morphologically indistinguishable from the bodies seen in direct smears from the lesions, and that, apparently, these bacteria are members of the group of which *B. mucosus capsulatus* is the type.

Their relationship to the production of the disease, however, awaits extensive experimental inoculation studies of which, as yet, there are not very many reports.

Cornwall and Peck,⁶ however, have recently described an organism which they believe does not belong to the *B. mucosus capsulatus* group and which, in old cultures, reproduces exactly the morphology of the Donovan bodies.

In a second paper⁷ they report inoculation experiments on rabbits receiving the same organism from the lesions produced, and Goldzieher and Peck⁸ continuing studies on the same organism report studies supporting their belief in the etiologic relationship of this organism, for which they propose the name *Bacillus venereogranulomatis*, by complement-fixation and allergic tests as well as animal inoculation.

The question remains an unsettled one worthy of extensive study.

REFERENCES

- ¹Fox, H. Granuloma Inguinale. Its Occurrence in the United States, Jour. Am. Med. Assn., Nov. 27, 1926, LXXVII, 1785.
- ²Conyers, J. H., and Daniels, C. W. Lupoid Form of the So called Grain Ulceration of This Colony (British Guiana), Brit. Guiana Med. Ann., 1896, VII, 13.

- *Donovan Indian Med. Gaz., 1905, xl, 414
 *Lynch, K. M. Granuloma Inguinale, Jour Am Med Assn, 1921, lxxxvii 925
 *Campbell M. F. Granuloma Inguinale, Jour Am. Med. Assn, 1921, lxxvi, 648
 *Cornwall L. H., and Peck S. M. Three Cases of Granuloma Inguinale etc, Proc New York Path. Soc., 1925 xxv, 5, 42
 *Cornwall, L. H. and Peck, S. M. Etiology of Granuloma Inguinale, Arch Dermat and Syph 1925, xii, 613
 *Goldzieher, M., and Pick, S. M. Arch. Path and Lab Med, 1926, i, 4, 511

—R. A. K.

Some Recent Contributions on the Treatment of Pneumonia

THE last two or three years may be characterized as a period in which we have been becoming more intimately acquainted with the pneumococcus as a germ. Careful bacteriologic, immunologic and chemical studies of the pneumococcus cell are being made and while many of the observations recorded have no direct or apparent bearing on the treatment of pneumonia, the result will be an accumulation of knowledge, some of which will be applied in practical therapeutics. This intensive study is being carried on particularly by Avery, Dochez, and their coworkers at the Rockefeller Institute, by Felton and Bailey at Harvard and by Falk and Jacobson at the University of Chicago.

Work at the Rockefeller Institute indicates that the fixed type pneumococci may be divided into two categories *type specific* or S strains and degraded or R strains. Both groups are present in all three of the fixed types. The type specific pneumococci retain their capsules. The degraded ones have lost them. The type specific antigens appear to be associated with these capsules and are carbohydrate, polysaccharide, in nature. Remann finds that immune sera for the degraded R strains cross agglutinate. A serum immune to a Type II R strain will also agglutinate other fixed type R strains. It will not agglutinate fixed type encapsulated S strains, not even of Type II. Apparently in immunization against pneumococci two varieties of agglutinins are produced, a general one for the pneumococcus protein and a specific variety against the type specific antigen. While the R immune sera do not agglutinate the type specific pneumococci they do precipitate the free protein of these S strains after autolysis. Here the pneumococcus protein has apparently been liberated from the type specific antigen.

The R strains are serologically identical. They evoke *species-specific* antibodies and not *type specific* antibodies.

The recognition of type specific and degraded pneumococci explains confusing cross reactions which have been observed and might conceivably have some bearing on the varied results that have been reported in the literature following treatment with Type I immune serum. A Type I serum produced with degraded Type I pneumococci will have little or no effect against a type specific Type I pneumococcus.

Sia finds that the specific soluble substance from a fixed pneumococcus increases the virulence of an otherwise avirulent pneumococcus. This specific soluble substance is polysaccharide in nature and is probably related to

or the same as the type specific antigen previously discussed. Pneumococci, either naturally endowed with this substance or reinforced by its addition, become more virulent.

Within the last few years there has been no outstanding alteration in the serum treatment of lobar pneumonia. The results obtained with Type I serum at the Rockefeller Institute still stand, although certain workers have not obtained as convincing results. Perhaps the most outstanding requisite for success is serum administration at as early a date in the disease as possible. More recently Gay and Chickering have produced a concentrated Type II serum which carries with it some promise of results.

Polyvalent, immune sera are of value only in Type I pneumococcus infection, for they do not contain a sufficiently potent concentration of Types II and III antibodies to be of value.

Huntoon has separated the antibodies from the serum proteins. The separation of the immune body from the other serum constituents is naturally an ideal to be attained. He prepares a polyvalent serum and by exposing it to contact with pneumococci effects combination between the antibodies and the antigen. The antigen has become sensitized. The antibody has become absorbed. The bacteria are then centrifuged and washed with salt solution until the serum is entirely removed. The antigen-antibody combination is next emulsified and an appropriate amount of alkali added. The mixture stands overnight, during which time the antibody is set free in great part from combination with the antigen. The latter is then thrown out by centrifugation.

The supernatant fluid contains the antibody with agglutinins and probably a slight portion of antigen still attached. This is further purified and finally filtered through porcelain. There is practically no protein in the solution.

Antibody solution injected intravenously has produced occasional severe reactions which have in one or two instances terminated fatally. For this reason subcutaneous administration was substituted. Oliver and Stoller report then results with the subcutaneous administration of antibody solution. They found that in a study of twenty-three cases two had mild febrile reactions, all had local pain and one developed what appeared to be an extensive cellulitis. Only four experienced subjective improvement and but three displayed objective improvement. The remainder were not benefited. They concluded that pneumococcus antibody solution as prepared at that time (1925) is of less value in the treatment of Type I pneumonia than is Type I serum. It does not prevent extension to other lobes. Their best results were obtained in Type IV infection where the mortality was 10 per cent.

They found that subcutaneous administration did not sterilize the blood stream. This apparently is due to the fact that antibodies either do not appear, or appear but slowly within the blood stream.

Huntoon therefore has experimented further with intravenous administration. He finds that the severe reactions are apparently usually associated with extraneous bacterial contamination. This has been greatly obviated by manipulation at low temperatures. He has also perfected a method of anti

body concentration. It can now be concentrated about 40 times with the result that the dose is 5 or 10 c.c. intravenously instead of the former 50 to 100 c.c.

Baldwin and Cecil report promising results in the treatment of Types I and II pneumonias, with Felton's concentrated serum.

The vaccine treatment of pneumonia is, as usual, before us for consideration. Rosenow and Hektoen in 1913 recommended the administration of partially autolyzed pneumococcus antigen. This appears not to have been followed up in recent years. Alexander Lambert reports treatment with a mixed bacterial vaccine containing in each c.c. 200 million Pfeiffer bacilli, 100 million pneumococci, 100 million streptococci, 200 million Micrococcus catarrhalis and 200 million each of Staphylococcus albus and aureus. There are in all 160 strains of bacteria. He injected from 1 to 2 c.c. of this vaccine intramuscularly every six hours until the temperature had reached 99 and then every twelve hours for two or three days, and finally once daily until symptoms had entirely subsided. He reported no reaction from these treatments. Two hundred twenty one cases so treated were compared with 286 control cases observed during the same season. The mortality among the treated was 19 per cent as contrasted with 37 per cent among the controls. When treatment was started within the first forty eight hours the death rate was reported as 5.8 per cent within the first seventy two hours as 9.8 per cent. He records a diminished severity rather than a shortening of the course of the disease, following this mode of treatment.

It is a matter of ancient observation that patients with lobar pneumonia are more comfortable when in the open air. Oxygen treatment received its first impetus following its application in 1917 by Haldane in the treatment of acute pulmonary edema. Various apparatus have been devised for the oxygen administration, some simple some intricate some apparently efficient others inefficient. Barach delineates the present status of oxygen therapy. For satisfactory results the inspired air should contain from 30 to 60 per cent oxygen. It is not safe to breathe for a long period air containing over 70 per cent oxygen. Best results are to be anticipated when the oxygen concentration is between 40 and 50 per cent. Below 30 per cent oxygen is ineffective. The ordinary funnel method delivers oxygen in a concentration of about 24 per cent, scarcely 5 per cent increase over atmospheric concentration. The proper use of the nasal catheter will deliver oxygen into the nasopharynx at a concentration of 30 per cent. This is when the oxygen is being run at the rate of about 2 liters per minute. With the 'bubble method' usually but 1 liter is delivered per minute.

There are many potential sources of error in nasal catheter administration. The catheter may be in the anterior portion of the nose not in the nasopharynx. It easily becomes clogged and should therefore have several perforations in the tip and should be cleaned every four hours. The patient may breathe entirely through the mouth. Too abundant oxygen flow may be irritating to the patient.

The Barach rebreathing apparatus, equipped with soda lime for the absorption of the expired carbon dioxide, will deliver 40 per cent oxygen con-

centration when the gas is being run at a rate of 1 liter per minute. This results in a distinct saving of oxygen.

The tent methods are apparently most efficient, delivering from 40 to 60 per cent concentration. Guedal has devised a simple, cheap oxygen tent made with barrel hoops cut in two and arranged criss-cross to serve as supports and covered with a single layer of muslin. This is placed over the patient's head and oxygen is delivered through a tube the end of which is in the neighborhood of the patient's face. No provision is made for removal of carbon dioxide, although a small aperture is sometimes left along the base for ventilation. Delivering 3 liters of oxygen per minute, the author maintains a concentration under the tent of about 35 per cent. His criterion for administration is the extent of cyanosis of the finger nails. Oxygen is given until the nails are no longer cyanosed. The oxygen flow is then adjusted at such a rate as will keep the nails free from blueness.

In this work commercial oxygen in high pressure cylinders is more economic than low pressure medicinal oxygen. It may be purchased in 110 or 220 cubic foot cylinders to which are attached a pressure gauge so graduated as to indicate the flow of oxygen in liters per minute. The expense with the Guedal apparatus, which is rather extravagant of oxygen, using on an average of 3 liters per minute, runs from \$6 to \$8 per day.

No extravagant claims are made for oxygen in reducing mortality from lobar pneumonia. Barach characterizes the treatment as supportive but not curative. It reduces the cyanosis, dyspnea and restlessness. The respiration and pulse are often favorably influenced and delirium if present is usually decidedly lessened.

Diathermy is a relatively recent departure in pneumonia therapy. The pneumococcus will not long stand a temperature of 106° or over. As is well known the application of diathermy increases the temperature of the tissue between the electrodes. Stewart claims for diathermy an improvement in the circulation through the hepatized lobe, improvement in coronary circulation and increased phagocytic activity. He finds that after diathermy the pneumonia patient experiences a change in the character of the respiration rather than in the rate, the respiration being deeper and freer, due perhaps to the diminished pleural pain or perhaps in part to the other changes attributed to the treatment. He states that the relief from cyanosis is constant, ascribing it to better functioning of the right ventricle and perhaps to better aeration in the lungs. The temperature falls by lysis rather than by crisis. Diathermy so far has not prevented extension into other lobes. Stewart describes a mortality of 15 per cent as contrasted with 43 per cent in control cases. There were 254 in the former group and but 31 in the latter. The majority were not typed. Walsh observed a 12.3 per cent mortality in 95 diathermy treated cases and 20.3 per cent among 59 who did not receive this treatment.

The drug treatment of lobar pneumonia is practically in statu quo as compared with the last few years. Optochin, ethylhydrocuprein, described by Moore in 1915, has developed little farther. No literature of great importance has appeared on this remedy within the last two years. This quinine

derivative appears to be directly pneumococcal but is too toxic, producing amblyopia in several cases, permanent contraction of the visual fields in a few and permanent blindness in one. Otherwise this remedy held considerable promise, the pneumonia mortality being around 10 per cent.

Digitalis continues to hold its place. As in the past there is no unanimity as to whether it should be given throughout the course of the illness or withheld until specific indications arise. With the methods for rapid digitalization now available its administration to an otherwise normal heart is as a rule not necessary. Where evidence of myocarditis exists, digitalis should be instituted without delay. Lawrence studying 1000 cases at Camp Devens found x ray evidence of enlargement of the heart early in pneumonia. Robinson at the Atlanta meeting of the Southern Medical Association reported experimental evidence showing that digitalis diminishes the size of the heart thereby increasing its output and its efficiency. In summarizing the digitalis problem we may say that the proper time for its administration remains optional.

Mercurochrome intravenously or intraperitoneally has been recommended for pneumonia in children. Hoppe and Freeman treated 23 cases of lobar and bronchopneumonia with mercurochrome in the usual dosage. The mortality among these children was 8.5 per cent as against 39 per cent in the controls. The duration of illness in the former was 6.5 days as contrasted with 16.5 days in the latter.

Spectacular, if not extravagant claims are made by Nott for the potassium permanganate treatment of lobar pneumonia. Two grains of the pure chemical are dissolved in one and one half pints of warm water and this is given slowly per rectum in three or four ounce quantities every two and one half to four hours for the first twenty four to thirty six hours. After this it is administered twice daily for three days then once a day for three days. The course of the illness naturally determines the frequency and length of administration. Forty cases have been so treated with a 5 per cent mortality. Nott claims for this method a remarkable sedative action, the clearing up of blood from the sputum within a few hours after its first administration and a rapid temperature fall by lysis. He makes no attempt to explain the phenomenon. If the results are as good as the author believes they are deserving of substantiation with more carefully controlled clinical observation. He also administers thyroid extract by mouth but doubts whether this is a factor in the good results.

In reviewing the advantages claimed for these various therapeutic procedures, in terms of the mortality rate the great variation in both treated and control cases is evident. In view of the differing severity of the disease at different seasons and in different local prevalences it is necessary each time to run a control series treated under analogous circumstances. Even then, however pneumonia is a disease with so many varying factors and complications that few series treated by any one method are sufficiently large to eliminate the influence of these variables.

Thus the type of pneumococcus infection a factor of great importance is sometimes not mentioned. Some of the reports which we have reviewed

make no mention of the causative organism. Type I pneumococcus is associated with a rough mortality of 25 per cent, Type II of 32 per cent, Type III of 45 per cent and Type IV of 16 per cent. A different relative proportion of type infections between the test series and the control series will vitiate the results. Probably the best figure that we have on general pneumonia mortality is that of Wells who reports a 20.4 per cent death rate in 465,400 cases of pneumonia. Here the number is sufficiently large so that the complicating factors mentioned have become well nigh negligible.

The great discrepancy in the death rates reported above makes comparison difficult or impossible. This brings us to the conclusion that there is at present no "all around" specific for lobar pneumonia. Each case must be individualized. The basic principles are still important: rest, both mental and physical, abundant fluids, a light diet, particular care to avoid abdominal distention, digitalis where indicated, caffeine as a respiratory stimulant occasionally, abundant fresh air, and a careful watch for complications. Of these probably the most important is rest, natural if possible, induced if necessary.

Beyond this the doctor treating the individual case will be interested not so much in a series mortality as in selecting those of the more recent therapeutic measures which are calculated to give the patient greater subjective and objective relief from the individual symptoms as they arise. Some of the measures described above will fall within this category.

REFERENCES

- Reimann, Hobart A. Serological Relationships of Type Specific and Degraded Pneumococci, *Jour Exper Med*, 1926, *xliv*, 107.
- Oliver, Wade W., and Stoller, E. A. Notes on the Therapeutic Value of Pneumococcus Antibody Solution Subcutaneously Administered in Lobar Pneumonia, *Arch Int Med*, 1925, *xxxv*, 266.
- Huntoon, F. M. Pneumococcus Antibody Solution, *JOUR LAB AND CLIN MED*, 1926, *vi*, 759.
- Lambert, Alexander. Use of Mixed Stock Vaccines in Pneumonia, *Jour Am Med Assn*, 1926, *lxxvii*, 56.
- Barach, Alvan L. Methods and Results of Oxygen in Pneumonia, *Arch Int Med*, 1926, *lxvii*, 186.
- Geudal, Arthur E. Oxygen Therapy in Pneumonia, *Jour Am Med Assn*, 1925, *lxxxiv*, 1490.
- Walsh, Groesbeck F. Diathermy and Treatment of Pneumonia, *South Med Jour*, 1925, *xviii*, 500.
- Stewart, Harry E. Diathermy with Special Reference to Pneumonia, ed 2, 1926, Hoeber.
- Hoppe, Lewis D., and Freeman, Wm. T. The Treatment of Pneumonia in Children with Methylenechrome Intravenously, *South Med Jour*, 1925, *xviii*, 784.
- Sia, Richard H. P. Studies on Pneumococcus Growth Inhibition VI, *Jour Exper Med*, 1926, *lvi*, 633.
- Nott, Herbert W. The Thyroid and Manganese Treatment in Acute Pneumonia, *Brit Med Jour*, 1926, *lii*, p. 109.
- Rosenow and Hektoen. *Jour Am Med Assn*, 1923, *xl*, 2203.
- Baldwin, H. S., and Cecil, R. L. The Rationale of Specific Therapy in Pneumococcus Pneumonia, *Jour Am Med Assn*, Nov 20, 1926, *lxxxvii*, 1709.

—W T V

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN M.D. Editor in Chief
Medical Arts Building
Richmond Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E JACKSON M.D.
University of Cincinnati Cincinnati

Physiology

J J R. MACLEOD M.B.
University of Toronto Toronto

Pathology

PAUL G WOOLLEY M.D.
Los Angeles

Tuberculosis

GERALD B WEBB M.D.
Cragmor Sanatorium Colorado Springs

Clinical Pathology

W C MacCARTY M.D.
Mayo Clinic Rochester Minn.

HAROLD D CAYLOR M.D.
Mayo Clinic Rochester Minn.

Biochemistry

VICTOR C MYERS Ph.D.
State University of Iowa Iowa City

Experimental Medicine

RUSSELL L HADEN M.D.
University of Kansas Rosedale

Immunology

JOHN A. KOLMER M.D.
University of Pennsylvania
Philadelphia

ROBERT A. MILDREFF M.D.
Atlantic City N J

Bacteriology

A PARKER HITCHENS M.D.
Bureau of Science, Manila, P I

Surgery

DEAN LEWIS M.D.
Johns Hopkins University Baltimore

Internal Medicine

GEORGE R. HERRMANN M.D.
Tulane University New Orleans

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY 3523 25 PINE BLVD ST LOUIS

Entered at the Post Office at St Louis Mo as Second Class Matter

The New Fifth Revised Edition of a Popular Book

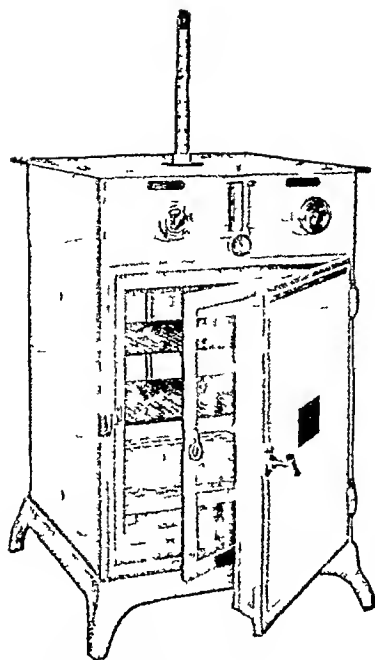
Physiology and Biochemistry in Modern Medicine

Over 1100 pages 6 1/2 x 9 1/2 with 250 illustrations including half tones and line drawings and 9 color plate. Price with cloth binding \$11.00

BY J J B MACLEOD M.B. LL.D. D.Sc. F.R.S.

Professor of Physiology in the University of Toronto. Formerly Professor of Physiology in the Western Reserve University

Assisted by H.C.I. and L.C. Redfield A.D. Taylor J.M.D. Olin and by others.



Freas Incubator No 26

Your Incubator Must be Accurate

Just as with your laboratory worker your incubator must be capable of performing the assigned tasks accurately

Accuracy in an incubator is its ability to maintain the temperature constant continuously

This is why the Freas Incubators are used in the leading laboratories

The Thermo Electric Instrument Co.
1215 South Grove Street, Irvington, N. J.

FREAS

ELECTRIC INCUBATORS

was repeated by using asparagin medium instead of broth, the arrangement being otherwise the same. The results were identical with those obtained with broth.

II A great majority of bacteriologists still use D'Herelle's method for the interpretation of their results with bacteriophage. To avoid objections that the above experiment was carried out with large quantities of phage, the following experiment was made. Ten tubes were filled with 10 cc of 1 per cent dextrose broth. This sugar concentration was selected due to the fact that this amount was sufficient to produce high acidity of the medium while the bacterial growth within twenty four hours was quite heavy. In addition the first tube obtained 1 cc Shiga phage, the second tube obtained 0.1 cc phage, the third 0.01 cc phage, etc. A duplicate series with plain broth was prepared in the same manner. One tube with dextrose broth and bacteria and another tube with plain broth and bacteria served as controls. All tubes were seeded with one loop of Shiga bacilli. The lytic power of the phage tubes was tested before seeding with bacteria. After twenty four hours incubation at 37° C the filtrates of these tubes were again tested for the presence of phage. Usually the control tube with dextrose showed a heavier bacterial growth than the tube with plain broth. A partial or complete disappearance of phage was observed in tubes containing dextrose and smallest amounts of phage while the corresponding tubes in plain broth showed phage in small quantities. D'Herelle and many others have repeatedly observed that small amounts of phage favor the development of resistant bacteria. After twenty four hours incubation the bacteria in all tubes were tested on plates against the phage. It was found that the tubes where the phage disappeared always contained resistant bacteria. We have observed very often, that sugar containing media show a heavier bacterial growth than those without sugar. This observation can easily be explained due to the fact that the addition of a suitable sugar to the medium increases its nutritive power. Such increased bacterial growth causes a disproportion between phage and bacteria, and leads to the formation of resistant bacteria. This second experiment seemingly contradicts the first. The results of this experiment indicate that the phage are destroyed only in comparatively small quantities.

Such a conclusion would be perfectly in accord with D'Herelle's conception about the relation between resistant bacteria and phage. He assumes that resistant bacteria destroy phage.

III Da Costa Cruz⁹ and some others found that the phage enter the dead bodies of the susceptible bacteria. This fact induced us to consider the possibility that the phage enter the bodies of resistant bacteria and remain there inactive. Such an occurrence in a culture containing phage and a large number of resistant bacteria would distinctly decrease the amount of the phage. If the quantity of phage were large the decrease could be easily overlooked. If the quantity were small it is possible that all the phage enter the bodies of the resistant bacteria and the filtrate of the culture will not contain any phage when tested on plates. It is generally accepted as a fact that the multiplication of phage occurs at the expense of the susceptible bacteria. Bacteriophage which enter the resistant bacteria would not be able to multiply.

In order to determine whether the phage are destroyed by resistant bac-

our phage do not weaken at $P_H 4.8$. At $P_H 4.4$ the activity is decreased about 50 per cent or more and at $P_H 4.0$ the phage are completely inactive and do not recover any more.

D'Herelle's suggestion to use glucose broth for isolation of bacteria from bacteriophage would be very useful and simple, if two conditions were fulfilled: (1) the acidified medium should not inhibit bacterial growth, (2) this acid medium should completely destroy phage. We thought it worth while to make a detailed analysis of this method for isolation of bacteria. Several factors should be considered: (1) the composition, the sugar content, and the reaction of the medium, (2) the intensity of bacterial growth and the development of resistant bacteria, (3) the influence of the H-ion concentration on the reaction of phage and the relation of phage to resistant bacteria, (4) the use of trypsin for detection of phage inside of bacterial bodies, (5) the influence of a prolonged incubation on the behavior of bacteria and phage, (6) a reliable method for reading and interpretation of the obtained results.

EXPERIMENTS

I. In order to determine the influence of the sugar concentration on the reaction of the medium, the bacterial growth, and the lytic principle, the following experiment was carried out. To a series of tubes containing 10 c.c. of broth, dextrose was added in various concentrations (0.01 per cent, 0.1 per cent, 1 per cent and 5 per cent). The reaction of all the tubes was set at $P_H 7.0$. To each tube Shiga phage were added in a sufficiently large quantity to cause complete lysis (1 c.c.). Each tube was then seeded with a loop of Shiga bacilli. A second series of tubes with the same sugar content, as the first series, did not contain bacteriophage. As controls served: (1) a tube with plain broth and the above amount of phage, (2) a tube with plain broth and bacteria, (3) a tube with plain broth, bacteria and phage. All the tubes were kept for twenty-four hours at $37^\circ C$. The tubes of the first series were perfectly clear with few exceptions, similarly the first and third controls. The tubes of the second series were more cloudy than the second control. Transfers were made on plates from the tubes of the first series and five drops of phage were added to each plate. Transfers of the cloudy tubes of the first series showed resistant bacteria on plates. The contents of the phage tubes were pulled through the Berkefeld filter and tested on plates for the presence of phage. Filtrates of every tube of the first series showed phage on plates. After the incubation, the H-ion concentration of all the tubes was determined. Control No. 1 did not show any changes, controls Nos. 2 and 3 showed only slight ones. Tubes seeded with bacteria only, showed a gradual increase of acidity corresponding to the sugar content. The highest acidity obtained was $P_H 4.7$, which could be observed in tubes with 1 per cent glucose, tubes with a higher glucose content than 1 per cent did not show a higher acidity. Tubes with bacteria and phage, showed much less acidification of the medium. In tubes with phage and a large number of resistant bacteria, the H-ion concentration was almost the same as in the corresponding tubes without phage. In all tubes which contained bacteriophage before seeding with bacteria, the lytic principle could be found in the filtrates after the incubation. A complete destruction of phage was not found in a single tube. This experiment

was repeated by using asparagin medium instead of broth, the arrangement being otherwise the same. The results were identical with those obtained with broth.

II A great majority of bacteriologists still use D'Herelle's method for the interpretation of their results with bacteriophage. To avoid objections that the above experiment was carried out with large quantities of phage, the following experiment was made. Ten tubes were filled with 10 cc of 1 per cent dextrose broth. This sugar concentration was selected due to the fact that this amount was sufficient to produce high acidity of the medium while the bacterial growth within twenty four hours was quite heavy. In addition the first tube obtained 1 cc Shiga phage, the second tube obtained 0.1 cc phage, the third 0.01 cc phage, etc. A duplicate series with plain broth was prepared in the same manner. One tube with dextrose broth and bacteria and another tube with plain broth and bacteria served as controls. All tubes were seeded with one loop of *Shiga* bacilli. The lytic power of the phage tubes was tested before seeding with bacteria. After twenty four hours incubation at 37° C the filtrates of these tubes were again tested for the presence of phage. Usually the control tube with dextrose showed a heavier bacterial growth than the tube with plain broth. A partial or complete disappearance of phage was observed in tubes containing dextrose and smallest amounts of phage while the corresponding tubes in plain broth showed phage in small quantities. D'Herelle and many others have repeatedly observed that small amounts of phage favor the development of resistant bacteria. After twenty four hours incubation the bacteria in all tubes were tested on plates against the phage. It was found that the tubes where the phage disappeared always contained resistant bacteria. We have observed very often, that sugar containing media show a heavier bacterial growth than those without sugar. This observation can easily be explained due to the fact that the addition of a suitable sugar to the medium increases its nutritive power. Such increased bacterial growth causes a disproportion between phage and bacteria, and leads to the formation of resistant bacteria. This second experiment seemingly contradicts the first. The results of this experiment indicate that the phage are destroyed only in comparatively small quantities.

Such a conclusion would be perfectly in accord with D'Herelle's conception about the relation between resistant bacteria and phage. He assumes that resistant bacteria destroy phage.

III Dr Costa Cruz⁹ and some others found that the phage enter the dead bodies of the susceptible bacteria. This fact induced us to consider the possibility that the phage enter the bodies of resistant bacteria and remain there inactive. Such an occurrence in a culture containing phage and a large number of resistant bacteria would distinctly decrease the amount of the phage. If the quantity of phage were large the decrease could be easily overlooked. If the quantity were small it is possible that all the phage enter the bodies of the resistant bacteria and the filtrate of the culture will not contain any phage when tested on plates. It is generally accepted as a fact that the multiplication of phage occurs at the expense of the susceptible bacteria. Bacteriophage which enter the resistant bacteria would not be able to multiply.

In order to determine whether the phage are destroyed by resistant bac

teria or enter these bacteria and remain there inactive, but alive, the following experiment was carried out. We prepared 10 tubes, each with 20 cc of 1 per cent dextrose broth, decreasing amounts of phage (2 cc, 0.2 cc, etc.), and one loop of Shiga bacilli. Three drops were removed from each tube and tested on plates. The tubes and plates were then incubated for twenty-four hours at 37° C. After the incubation, the tubes were heated for one hour at 55° C and the reaction adjusted to P_H 7.5. Fifteen cc of the contents of the tubes were pulled through the Berkefeld filter and tested on plates for the presence of the lytic principle. To the filtrates and remaining contents of the cultures, trypsin was added at 1 per cent. The trypsin was previously tested for the presence of phage, and the results were negative. The trypsin-containing tubes were then incubated for forty-eight hours at 37° C, pulled through the Berkefeld filter and tested on plates. The results showed that the filtrates of cultures exert the same lytic activity before and after tryptic digestion. The filtrates of digested cultures show a stronger phagic activity than the corresponding digested and undigested filtrates, this difference increases toward the lower dilutions. In some low dilutions, the filtrates of cultures before and after tryptic digestion are perfectly negative, while the corresponding filtrates of digested cultures show a more or less distinct phagic activity. This increase of phagic activity in the filtrates of digested cultures can be explained only by the fact that the phage are liberated by tryptic digestion from the bacterial bodies. The tubes with negative filtrates contained a large number of resistant bacteria, the degree of resistance was tested on agar-plates by adding from cultures one loop of bacteria to increasing amounts of phage. The absence of phage in plain filtrates and the presence in filtrates of digested cultures with resistant bacteria indicate that the phage are in this instance liberated from the bodies of the resistant bacteria. These findings justify the following conclusions: (1) resistant bacteria do not destroy phage, (2) bacteriophage which enter the bodies of resistant bacteria can be liberated by tryptic digestion, (3) phage liberated from resistant bacteria are not derived from trypsin and possess the property of regeneration as any other phage, (4) filtrates of cultures with a large number of resistant bacteria and a small amount of phage do not contain any phage, (5) the same cultures digested again show phage, (6) trypsinized culture filtrates cannot contain phage, if they did not contain it previous to the digestion. D'Herelle's classification of bacteriolysis in two acts is therefore justified. The first act occurs regardless of whether the bacteria are susceptible or resistant, but belong to the same susceptible strain, in the second act, the actual lysis occurs only with susceptible bacteria.

IV These findings induced us to consider that the bacteriophage may possibly enter the bodies of any bacteria and there produce lysis if the bacteria are susceptible, and remain inactive, but alive, if the bacteria are resistant. To test these possibilities, the experiment was worked out in a manner similar to the foregoing. Instead of the resistant bacteria of the susceptible strain, *Bacillus subtilis* and *Bacillus pyocyaneus* were employed. The results were entirely negative. There was no difference between the filtrates of cultures before and after the incubation and between the filtrates of cultures before and after tryptic digestion. These experiments were repeated several times, the results were always negative.

V In the following experiment the influence of time was studied more closely. It was possible that a twenty four hour incubation was not sufficient to test completely the phage for the resistant bacteria. To ten flasks with 90 cc of 1 per cent dextrose broth, phage was added in decreasing amounts. To the first flask, 10 cc of Shiga phage were added and mixed with the broth. 10 cc from the first flask were transferred to the second flask and mixed with the broth, each succeeding flask thereby containing ten times less phage. In this manner the same concentrations of phage were prepared as described in previous experiments. Before seeding with bacteria the phage dilutions were tested on plates. All flasks were seeded with 1 cc of a twenty four hour old culture of Shiga bacilli. After a twenty four hour incubation 10 cc were removed from each flask, filtered through the Berkefeld filter and tested on plates. Ten cc from each flask were subjected to a forty eight hour tryptic digestion, pulled through the Berkefeld filter and tested on plates. This procedure was repeated on the fourth day and after one and two weeks. The filtrates of the cultures were also digested by trypsin. The H ion concentration of every flask was at the same time determined. The obtained results show that the highest degree of acidity in a 1 per cent dextrose broth is obtained within twenty four hours, and that a longer incubation does not produce any further changes. Similar were the changes in the lytic activity of phage. Bacteriophage, which disappeared after twenty four hours, could be liberated after two weeks as easily as after a twenty four hour incubation. A prolonged incubation in a sugar medium has therefore no influence on the purification of bacteria.

Sugars which are not decomposed by the bacterial ferments have no influence on the development of resistant bacteria, the results are the same as in plain broth. These observations were made previously by D'Helelle and some other authors.

VI We found it worth while to determine what changes would occur on the phage if the initial reaction of the sugar medium would be higher than P_H 7.0. Six series of phage dilutions were prepared, one with plain broth, the other with 1 per cent dextrose broth at P_H 7.0. In the same manner phage dilutions were prepared at P_H 8.0 and P_H 9.0. All these tubes were seeded with typhoid bacilli and kept for twenty four hours at $37^\circ C$. The H ion concentration and the phage content were determined before and after the incubation. At P_H 8.0 and P_H 9.0 there was no difference between phage dilutions in plain broth and in dextrose broth. The bacteriolysis was quite regular. The differences between the sugar free and sugar containing phage dilutions at P_H 7.0 were the same, as already described. The findings indicate that these changes in the behavior of bacteria and phage occur only in a medium slightly acidified by a fermentable sugar.

VII The filtrates of cultures were used in the above experiments for the interpretation of phagic activity. Simple transfers from cultures to agar plates were employed only for the detection of resistant bacteria. Very often such transfers give regular growth on plates, while the filtrates of the same cultures show strong phagic activity. Many contradictory findings can easily be explained by the fact that cultures were tested on plates instead of their filtrates. The following experiment gives an illustration of the great differences in results, if

cultures were used instead of filtrates. Ten tubes, each containing 10 cc of broth and decreasing amounts of typhoid phage were seeded with one loop of typhoid bacilli. These tubes were then kept for twenty-four hours at 37° C. After the incubation, one loop from each tube was transferred to plates. The contents of the tubes were then pulled through the Berkefeld filter and tested on plates. One loop of the filtrate and one loop of the original typhoid culture, used in this experiment, were spread on agar plates. It is obvious that to the plates of the second series more bacteria were added than to those of the first series. We could therefore expect less phagic activity on the plates of the second series. The results were contrary to our expectations and can be explained only as due to the appearance of resistant bacteria in the broth cultures. This explanation was verified in the following way. One loop from each culture was transferred to a plate and five drops of phage were added, two drops of the phage were sufficient to produce a perfectly sterile plate. Several plates showed a regular growth, while the corresponding plates from the filtrates showed a strong phagic activity. The results obtained indicate that the transfers of cultures on plates are not useful for the determination of the presence of phage, the results are more accurate if the filtrates of cultures are used instead of simple transfers, the filtrates represent the free phage in the culture fluid at the time of filtration.

DISCUSSION

Our experiments could not confirm the findings of Asheshov and Seiser, that the presence of glucose in the medium hastens the bacteriolysis. Some of our observations agree with those of D'Herelle: (1) nonfermentable sugars have no influence on the bacteriolytic process, (2) the bacteriolysis is not appreciably affected if phage in large quantities are present. Our findings disagree with those of D'Herelle concerning small amounts of phage. According to D'Herelle the acidification of the medium occurs before the regeneration of phage takes place. In almost all instances we observed regeneration of phage, even if incomplete, the regeneration was very often disturbed by the appearance of resistant bacteria. This fact could be noticed especially when the reaction of the medium changed slightly acid due to the fermentation of sugar. If the acid, formed by the decomposition of the sugar, was not sufficient to change the reaction of the medium, the regeneration of phage was regular. The produced acidity in the sugar medium was never higher than P_H 4.7, and this degree of acidity was not sufficient to destroy the phage completely. Very small quantities of phage were not found in the filtrates of cultures after the incubation but were recovered by tryptic digestion of the culture. Our findings concerning liberation of phage from resistant bacteria by tryptic digestion are contrary to those of D'Herelle. He claims that the phage do not enter the bodies of resistant bacteria but are destroyed by them. By using the same method we could not detect any bacteriophage in the bodies of nonspecific bacterial strains. It is generally accepted that the optimal activity of phage takes place within a certain range, even if they do not agree concerning the exact limits. We found that there is a decreased activity of phage below this range, the lower limit being P_H 4.8. Within this last range the above described phage phenomena

occur in a sugar medium the bacteria still show a comparatively good growth, while the phage are less active. Thus disproportion leads to the formation of resistant bacteria. A number of bacteriophage enter the bodies of resistant bacteria and remain there inactive. These bacteriophage do not pass through the Berkefeld filter, and if there are no free phage left in the culture, the Berkefeld filtrate will not contain any phage. The Berkefeld filtrate represents the free phage in a culture. The filtrate of a trypticized culture contains the free phage and those liberated from the bacterial bodies.

SUMMARY AND CONCLUSIONS

- 1 A fermentable sugar favors bacterial growth but not bacteriolysis
- 2 A nonfermentable sugar has no effect on bacterial growth or on bacteriolysis
- 3 Media, acidified by sugar fermentation have no visible effect on large quantities of phage
- 4 Media, acidified by sugar fermentation favor the development of resistant bacteria, if small quantities of phage are added
- 5 Media, acidified by sugar fermentation do not destroy bacteriophage, regardless of the concentration of sugar or length of incubation
- 6 If the sugar fermentation does not affect the reaction of the medium, the bacteriophage behave the same as in a sugar free medium
- 7 Bacteriophage enter the bodies of resistant bacteria of a susceptible strain but remain there inactive
- 8 Bacteriophage inside of bacterial bodies can be liberated by a forty-eight hour tryptic digestion
- 9 Bacteriophage do not enter the bodies of nonspecific bacteria
- 10 Filtrates of cultures should be tested on plates for interpretation of lytic activity. Simple transfers of cultures lead to erroneous conclusions on account of the appearance of resistant bacteria

REFERENCES

- ¹Asheshov, I. N. *Compt rend Soc de biol* 1929 lxxvii, 1341
- ²Seiser, A. *Arch f Hyg* 1923 xci, 139
- ³D Herelle, F. *The Bacteriophage and Its Behavior* Baltimore 1926 Williams and Wilkins.
- ⁴Ehava and Pozerski. *Compt rend Soc de biol* 1921 lxxxv, 139
- ⁵Scheidegger, E. *Ztschr f Hyg u Infektion krankh* 1923 xcix, 403
- ⁶Gratia, A. *Compt rend Soc de biol* 1921 lxxxv, 275
- ⁷Da Costa Cruz J. *Compt rend Soc de biol* 1924 xc, 236
- ⁸Arnold, L. and Weiss E. *Jour Immunol* 1926 xii, 393
- ⁹Da Costa, Cruz J. *Mem Inst Oswaldo Cruz*, 1925 xiv, 81

FAILURE OF THE MOUSE TEST TO DEMONSTRATE THE PRESENCE OF TYPE I PNEUMOCOCCUS IN SPUTUM AN UNUSUAL INSTANCE*

BY RUTH GILBERT, M D , AND C K DAVENPORT, A B , ALBANY, N Y

SO MUCH confidence has been placed in the mouse method for the determination of the type of pneumococcus present in sputum, that an instance in which this procedure failed to demonstrate the presence of Type I in a specimen containing both Types I and III may be of interest

The patient, a boy of fifteen years, complained of illness on June 12 Definite symptoms of lobar pneumonia did not develop until the fourteenth The specimen of sputum received on this date was insufficient for a Krumwiede precipitation test, Avery's culture medium and a mouse were, therefore, inoculated

A few hours after the first specimen was received, a second specimen, large enough to permit the performance of the Krumwiede precipitation test, was submitted The sputum coagulated readily, and the saline extract made from it was clear No precipitation, however, was obtained with any of the type pneumococcus sera This result was confirmed by repetition of the test

After approximately seven hours' incubation, there was sufficient growth in the Avery medium inoculated from the first specimen to warrant the performance of a precipitation test With the aid of an agglutinoscope a very faint reaction could be detected at the end of half an hour in the tubes containing Type I serum, both diluted and undiluted At the end of one and one-half hours the precipitate in these tubes was just visible to the unaided eye No reaction was obtained in the tests with Types II and III sera The tests were all made in duplicate

The physician in charge of the case, on receiving the results of these tests, decided to administer serum, but as the patient had in the past received large doses of diphtheria antitoxin, it was thought necessary to insure adequate desensitization before the large dose of antipneumococcus serum was given The desensitization was considered complete at eight o'clock, the following morning (June 15) At this time the boy's temperature was 105°, the pulse 110, and respiration 40 Fifty cubic centimeters of antipneumococcus serum were administered At 12 M the temperature had reached 105.6° At 4 P M the temperature was 104° At 8 A M an additional 95 cc of antipneumococcus serum were administered At midnight the temperature was 103.5° At 4 A M (June 16) it had dropped to 101.4° and continued to fall until midnight, when it had reached the normal temperature From then on, the pa

*Presented at the meeting of the Society of American Bacteriologists Philadelphia, Pennsylvania December 28-30 1926

From the New York State Department of Health Division of Laboratories and Research Albany

Received for publication March 9 1927

tient's recovery was rapid and uneventful, aside from a rash which developed on the nineteenth and gave him considerable discomfort for several hours.

On examination of the blood agar plates inoculated from the sputum and also from the Avery cultures, the predominating colonies were found to resemble those of pneumococci or green producing streptococci. Suspensions made from the growth washed from these plates were agglutinated definitely by Type I pneumococcus serum both diluted and undiluted and, much to our surprise, by undiluted Type III pneumococcus serum also. No colonies characteristic of Type III pneumococcus were observed on any of the plates. Of ten colonies fished from the plates before the growth was removed for the agglutination tests, four proved to be cultures of pneumococcus Type I and six of *Streptococcus viridans*.

The mouse that had been inoculated with this sputum was found dead at the end of forty eight hours. Agglutination, precipitation, and cultural tests demonstrated the presence only of Type III pneumococcus. These results seemed so unusual that a third specimen of sputum was requested on the sixteenth. A mouse inoculated with this specimen was found dead at the end of forty eight hours. Agglutination and precipitation tests made with the peritoneal fluid showed marked reactions only with the Type III antipneumococcus serum. Colonies of both Types I and III however developed on the plates inoculated from the heart's blood and peritoneal fluid.

It was thought possible that Type I pneumococcus from this case might not be pathogenic for mice. Five mice were therefore inoculated with 10 c.c. of a twenty four hour broth culture. Two died within twenty four hours, one after two days and two within four days. The presence of Type I pneumococcus was demonstrated in all five animals. The delayed death of some of the mice inoculated with these large amounts of culture would indicate that the virulence of the cultures for these animals was low.

Although this report covers an isolated case only, it seemed worth recording since the patient appeared to have been so definitely benefited by the serum treatment and the presence of Type I pneumococcus in his sputum would not have been demonstrated by the mouse method alone.

A STUDY OF THE MICRO-KAHN TEST IN SYPHILIS

A REPORT OF 2100 REACTIONS*

BY ROBERT A. KILDUFFE, A.M., M.D.,† AND W. W. HERSOHN
ATLANTIC CITY, N. J.

THE progress of any development in the history of medicine is by evolution rather than revelation, of which circumstance the gradual unfolding of the true relation of the complement-fixation test to the study of syphilis furnishes a striking example.

While the laborious and cooperative studies of a host of workers have culminated in the recognition of the complement-fixation test as the most delicate and constant *single* symptom of syphilis, the coincident appreciation of the inherent complexities of this test, which render it safe and reliable only in the hands of competent and well-trained serologic workers, has led to efforts either to simplify its technique or to find some equally reliable but technically simpler procedure.

To this end the application of flocculation or precipitation tests to the serologic study of syphilis has occupied the attention of numerous investigators, but not until the work of Kahn,^{1, 2} who demonstrated the essential necessity of extreme care in the concentration of the reagents (serum and antigen), as affecting, not only the occurrence but the constancy of such phenomena in syphilis, have these procedures assumed a position of practical importance.

It is unnecessary, for the purpose in view, to discuss the underlying principles of the Kahn test or to review in detail, or even in brief, the literature which has accumulated bearing upon its value. At present this discussion is concerned, apparently, not with the clinical and laboratory utility or non-utility of the Kahn test in syphilis, but with whether or not the Kahn test should supplant all other procedures and serve as the sole and exclusive serologic procedure in the study of syphilis, concerning which we shall have something to say later.

As has been consistently, and at times rather clamorously proclaimed, a salient feature of the Kahn test is its relative technical simplicity, and in the interests of technical simplicity various workers have endeavored to simplify still further the technique proposed by Kahn and to shorten the time and labor required for his test.

One of the most promising of the methods devised for this purpose is that of Kline and Young³ which has been reported upon by Kline, Littman and Mill⁴ who present a study of 2800 tests.

The purpose of this communication is to report a study of this procedure

*From the Laboratories of the Atlantic City Hospital.

†Director, Laboratories, Atlantic City Hospital.

Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, May 13, 1927, Washington, D. C.

in a series of 2116 tests in comparison with the Wassermann reaction upon the same sera

The comparison was undertaken at the suggestion of Dr Kline who not only supplied the necessary apparatus but very kindly demonstrated the technic to us

The sera examined comprised 1464 secured from the various departments of the Atlantic City Hospital and including sera sent for examination from patients outside the hospital and 652 from the Genitourinary Clinic of the Municipal Hospital for Contagious Diseases

For these latter specimens and also for accompanying data, we are indebted to the Chief of Clinic Dr C H de T Shivers and his Associate Dr C L Bossert, and their assistants

The complement fixation tests were all conducted—as is the routine practice in these laboratories—by the quantitative method described by Kolmer² which is now too well known and too widely used to necessitate a repetition of its technic

Because of the fact that during the demonstration of the micro test we learned that certain modifications had been made in the procedure as originally described—notably the discarding of the humidor—the technic of the slide test is presented below together with certain details which we have found to be of interest and value

TECHNIC OF THE SLIDE PRECIPITATION TEST

While ordinary microscopic slides suffice for the performance of four tests, it is more convenient and conserving of time and labor to use glass squares 3 inches square, and of the thickness of an ordinary microscopic slide, thus permitting 12 to 16 mounts at one time. Before use these glass plates are covered with bon ami paste which is allowed to dry when it is completely removed by polishing with a soft cloth

Immediately after use the paraffin rings are washed off by holding the slides under running scalding water. The slides are then covered with bon ami paste overnight and then cleaned the paraffin rings not being made until immediately prior to the test

An instrument is required for making the paraffin rings in which the tests are made on the slide. The one used by us was furnished by Dr Kline. Its preparation is thus described by Kline and Young³

A piece of No 28 soft iron wire 14 cm in length is twice wound tightly about a test tube 12.5 to 13 cm in diameter forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch from the free end

After removing the looped wire from the test tube, a piece of No 12 cotton thread about a yard long is started from the free end of the shaft after being fastened there by a single twist of the two free ends. Three long turns are made reaching the loop which is then tightly wound with the thread the winding being continued up to the free end of the shaft where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft and reshaped by working it against the

end of the test tube and the shaft is then fastened into a wooden holder such as a teasing needle

The paraffin rings are made by dipping the loop into smoking paraffin (about 120°) We have found that it is an advantage, after removing the loop from the paraffin, to touch it to the surface of an extra slide thus removing any excess of paraffin, and then rapidly press it lightly like a stamp on the slide to be used, thus making neat, regular rings of paraffin of approximately the same thickness and having a diameter of 11 to 12 mm

Pipettes—The pipette for delivering the serum is the usual 1 cc serologic type graduated in 0.01 cc

The pipette for delivering the antigen is a capillary pipette made from glass tubing about 6 mm in diameter, the delivery end being drawn to such a size that one drop of antigen dilution equals 0.015 cc The one used in this series was supplied by Dr Kline but they may be easily made

The vials used for preparing the antigen dilution are those recommended by Kahn and were supplied for our use by Dr Kline

The Antigen—The antigen for the test is that described by Kahn⁷ Those used in this series were originally supplied by Kahn and by Kline and later supplies were prepared by ourselves

After the antigen is finished it is placed in the ice chest for a day or two to precipitate the excess cholesterol and then filtered and kept at room temperature With this precaution it will remain crystal clear

The antigen titration is an important preliminary A series of dilutions of antigen and normal saline are made in the following proportions 1:1, 1:1.1, 1:1.2, 1:1.3, etc, these mixtures being made as described below, *using not less than 1 cc of antigen to start*

In one of the vials 1 cc of antigen is placed and 1 cc of normal saline in the other vial The salt solution is poured into the antigen and, *without waiting to drain the salt solution vial*, the mixture is poured back and forth five or six times This mixing should be rapidly done The result is a somewhat cloudy, opalescent solution which should be used at once

A similar procedure is performed with a mixture of 1 cc of antigen and 1:1 cc of normal saline and so on

These various antigen dilutions are now tested by the technic described below with a series of negative and positive sera to find the antigen dilution giving clear negatives with negative sera and well-marked positives with positive sera and the dilution thus found satisfactory is increased by 0.1 cc In other words, if the 1:1.1 dilution is satisfactory, 1 cc of antigen is diluted with 1.2 cc of normal saline for the test This dilution is then prepared and tested with a series of known negative and positive sera to check the titer

The Serum—These are obtained as for the Wassermann test, *care being taken that they do not contain blood cells or foreign particulate matter* Before use they are inactivated at 56° C Kline and Young recommend an inactivation period of thirty minutes Our routine is inactivation for fifteen minutes which was adhered to in this series

The Test—The improvised humidifier suggested in the original paper of Kline and Young has been found not to be necessary if the tests are performed

in a warm, humid atmosphere without drafts, the slides, pipettes, etc., not being chilled. The slides are placed upon a mat of heavy filter paper (one fourth to one half inch thick) or upon a piece of felt. If necessary the surrounding temperature may be raised by several Bunsen flames. *Not more than 12 to 16 tests are done at one time*

If more than forty five minutes are to be used in the performance of the test, it is better to make a new antigen dilution as these dilutions are not suitable for use over prolonged periods. A preliminary test of the antigen with several negative and positive sera is advisable.

With everything in readiness, 0.05 c.c. of serum (still warm from inactivation) is pipetted into one of the paraffin rings, twelve to sixteen sera being pipetted in series, using a fresh pipette for each serum.

With the capillary pipette one drop (equivalent to 0.015 c.c.) of antigen dilution is then added. If overdosage occurs through error in pipetting, the mixture overflows the paraffin ring, thus indicating the error.

After the addition of the antigen to all the sera (a matter of a second or two) the antigen and serum are *thoroughly* mixed by the flat end of a tooth pick, a fresh one being used for each serum. Following this mixture, which is rapidly done the slide is held by the edges between the fingers and rocked with a rotary motion for two minutes *by the watch*. If the paraffin rings have been properly made this agitation may be quite vigorous without spilling the serum antigen mixture.

When the two minute period is over the slide is placed upon the microscope stage and readings are made immediately with the low power of the microscope, using reduced light as in studying urine sediments and the coarse adjustment to penetrate all the levels of the field.

Readings—A negative reaction shows a clear, homogenous field without precipitate.

Positive reactions are indicated by the appearance of precipitates, recorded as plus minus, plus one, plus two, plus three, or plus four in accordance with the size of the particles and their number.

In doubtful cases or in case of technical mishaps, the test may be repeated and it is, perhaps, advisable that it be done routinely in duplicate using *different antigens*.

Kline and Young in accord with Kahn regard plus minus and plus one reactions as without diagnostic significance in which in the interests of the patient, we concur although, as will be shown later these are not always without some significance in cases of syphilis under treatment.

It is obvious that this technic is the acme of simplicity and exceedingly conserving of time. Nevertheless, it requires exceeding care in its minutiae to prevent the occurrence of false misleading or confusing reactions. Sources of error may be listed as follows:

- 1 Performance of the test at too cool a temperature
- 2 Chilled or cooled apparatus or sera
- 3 Use of sera containing foreign particulate particles
- 4 Dirty glassware

- 5 Improperly prepared or titrated antigen
- 6 Improper preparation of antigen dilutions
- 7 Neglect of thorough mixture and agitation of serum and antigen mixture
- 8 Delay in preparing or reading the tests (leading to disturbance of quantitative proportions by evaporation)
- 9 Personal equation in reading the results, *especially as regards the weakly reacting sera*
- 10 Errors of technique

The advantages of the micro-Kahn test as compared to the three tube Kahn test are that it requires less apparatus (no test tubes, rack, shaking machine, etc), less time and labor, less serum (one-seventh the amount is required for the micro-Kahn test), and the fact that the results are often more easily read.

All the micro-Kahn tests were made, read, and recorded before the Wassermann tests were completed.

RESULTS OF AUTHORS' SERIES

In reporting the relative total agreement of the micro-Kahn test with the Kolmer quantitative complement fixation test it is necessary to decide upon the status of the plus minus and plus one micro-reaction.

Of the 2116 sera tested 468 or 22 per cent were Wassermann positive. If, according to Kline and Young, the plus-minus and plus one micro reaction is regarded as without significance and thus in accord with a *negative* Wassermann, there were 299 positive micro-reactions (plus two or over) or 14 per cent positive reactions.

This is a total relative agreement between the two tests of 63 per cent.

If, on the other hand, the plus-minus and plus one micro-reaction is accorded significance and is thus in agreement with a *positive* Wassermann reaction, there were 425 positive micro-Kahn reactions or 20 per cent of the total number of sera or an agreement between the two tests of 90 per cent.

While we are quite willing to grant the difficulty of interpreting the diagnostic significance of the plus-minus micro-reaction, we are satisfied that the plus one reaction cannot be entirely disregarded and warrants a further study of the particular case and, further, that both plus-minus and plus one reactions are of some significance in the case of syphilis under treatment.

Procedures proposed for general adoption in the serologic study of syphilis must be applicable to unfavorable as well as to favorable situations. It is of interest, therefore, to consider the effect of physical characteristics of the serum as affecting their suitability for the micro-Kahn test.

Hemolyzed sera or those with a high icterus index do not interfere with the reaction. Chylous sera, or those contaminated with bacteria, molds, or particulate foreign particles are unsuitable as pseudoreactions resembling a plus minus or plus one reaction occur.

The micro-Kahn reaction with sera anticomplementary to the complement-fixation test is of interest, the results of 24 such sera being shown in Table I.

TABLE I
REACTIONS WITH ANTI-COMPLEMENTARY SERA

NO	MICRO-KAHN	SOURCE	REMARKS
1	0	Mum	Known lues
2	-		
3	-		
4	+		
5	+		same case specimens one week apart
6	---		
7	-		
8	0		
9	+		
10	+	Disp	No clots serum cloudy and contains molds
11	-	Hop	No evidence of lues
12	0		Same as 11 Wassermann later +4400 and Kahn negative
13	0		No evidence of lues
14	---		
15	0		Later Wassermann III No clinical evidence of lues
16	0		No evidence of lues
17	0		No evidence of lues
18	No reading possible		Corr blood hemolyzed
19	---		Lytic
20	No reading possible		Corr blood hemolyzed
21	0	Mum	Factorial treatment at two years
22	0		Lues primary lesion in treatment
23	0	Hop	Lues previous Wassermann positive
24	---		Lues late Wassermann positive

Several conclusions are obvious from a study of this table

a The micro-Kahn test gives a definite reading with a high percentage of anticomplementary sera this however is not invariably the case

b The micro-Kahn test may give a clear cut total negative reaction in the presence of syphilis

c The micro-Kahn test may give a doubtful positive reaction in the absence of syphilis

d Anticomplementary reactions and especially when they occur repeatedly and with fresh serum are followed by a positive Wassermann reaction in a definite number of cases

This is in accord with the feeling of many serologists that when persistent anticomplementary reactions are encountered which cannot be explained by technical factors the probability is in favor of syphilis

Interest also attaches to the results of the micro-Kahn tests upon cord bloods of which there were 97 in this series

Of these three were positive to both tests W = 42000 K = ---
W = 4100 K = --- W = 4444 K = ---

In all of these cases syphilis was proved There was therefore 100 per cent agreement of positive reactions

While all of the Wassermann reactions were clear cut negatives there were four (hemolyzed) sera with which micro-Kahn readings could not be made

The statistics thus far apply to the total sera tested Particular in

W = Wassermann test
K = Kahn test

terest attaches, however, to the 652 sera from the clinic because complete clinical data is available

Of these 652 sera 235 or 36 per cent were Wassermann positive. If all degrees of micro-Kahn reactions are regarded as true positive reactions, there were 208 or 32 per cent positive reactions, an agreement between the two tests of 88 per cent.

If, on the other hand, the plus-minus and plus one reactions are disregarded and classed as corresponding to negative Wassermann reactions then the total number of positive micro-Kahn reactions is 137 or 21 per cent, the agreement between the two tests falling to 58 per cent.

In view of the complete clinical check obtainable on these cases we feel that, if technical errors are eliminated, the weak micro-reactions cannot be dismissed as without significance. While we would decline to attribute diagnostic significance to the plus-minus reaction we believe its occurrence should lead to a repetition of both the Wassermann and the micro-Kahn tests as well as a thorough clinical search for evidence of syphilis, and, in the case of syphilis under treatment we believe the weak reactions can be classed as true positive reactions.

In the 652 sera under consideration there were 40 or 6 per cent in which the results of the two tests disagreed, the Wassermann being negative and the micro-Kahn plus two or more. Of these 40 sera, 36 were from syphilitic and 4 were from definitely nonsyphilitic patients in whom neither clinical nor serologic evidence could be obtained on repeated examinations.

In 30 cases the Wassermann reaction was negative and the micro-Kahn test was plus-minus or plus one. Of these evidence of syphilis was obtained in 24 cases. There were, therefore, 70 sera or 10 per cent in which the micro-Kahn test was positive in varying degree, 80 per cent of these sera being syphilitic.

There were 65 sera or 9 per cent in which the Wassermann reaction was positive and the micro-Kahn test negative, in all of these cases definite evidence of lues was obtained.

There was, therefore, 9 per cent of false negative micro-Kahn tests.

In 41 cases, not included in those noted in the preceding paragraph, the Wassermann reaction was positive and the micro-Kahn test plus-minus or one. All of these cases were syphilitic, the weak-micro-reactions, therefore, having a definite significance, what is of importance being the fact that in these cases giving weak micro-reactions the Wassermann reaction was never indeterminate but always distinctly positive.

In view of the relative proportion between the sera from the clinic for which clinical data were obtained, and the other sera, (1464) of the series, for which we were not always able to obtain clinical data, we feel that conclusions drawn from the smaller series may be applied without impropriety to the larger series. This is borne out by the statistics of the larger series which—as concerns agreements and disagreements—are quite comparable to those of the smaller.

Thus, 217 or 14.64 sera, or 14 per cent, were positive to the micro-Kahn test and 233 or 15 per cent were Wassermann positive, a total relative agree-

ment of 91 per cent. If the weak micro reactions are counted as negatives, the number of micro Kahn positive reactions becomes 162 or 11 per cent and the total relative agreement 78 per cent.

We believe the incidence of false negative and false positive reactions proved in the smaller series is applicable also to the larger series in which proof was not always available.

In common with other observers using precipitation tests based upon the principles expounded by Kahn we found a definite number of sera (281 or 13 per cent) in which there was disagreement between the two tests, the Kolmer being positive or the Kahn negative or vice versa.

Thus, there were 79 (39 hospital and 40 clinical sera) in which the Wassermann was negative and the micro Kahn plus two or more, an incidence of 3 per cent, and 53 (23 hospital and 30 clinic) in which the Kahn was doubtful (plus minus or one) and the Wassermann negative, an incidence of 2 per cent.

On the other hand there were 125 (60 hospital and 65 clinic) sera in which the Wassermann was positive and the Kahn negative, an incidence of 5 per cent, and 73 (32 hospital and 41 clinic) in which the Wassermann was positive and the Kahn doubtful an incidence of 3 per cent.

These figures are comparable to those of other workers, and furnish an obvious lesson to those who desire to see it.

The results of the comparison described above are comparable to those reported by Kline, Littman and Mill⁴ in a study of 2800 tests in their series an agreement of 94.9 per cent, and in our series an agreement of 88 to 90 per cent, being obtained. Kline, Littman, and Mill also report an agreement of 95.9 per cent with the regular three tube Kahn test a phase of the question not studied by us.

It thus appears that for those who desire to use the precipitation test as a check upon the Wassermann reaction or as an *additional means* for the serologic study of syphilis the micro Kahn test serves as a suitable method.

We desire to discuss also, however, a further aspect of this question of paramount importance, namely, can the precipitation reactions and especially those devised by Kahn, safely *supplant* the complement fixation test in the serologic study of syphilis?

The present literature concerned with the Kahn test does not appear to be interested in the determination of the value of this procedure as an additional and further means for the serologic study of syphilis but rather with a persistent agitation for its *exclusive* use for this purpose a sometimes even caustic demand that all other methods shall at once be discarded in its favor.

If it be objected that the results of our investigation cannot apply to those conducted upon the macroscopic method described by Kahn, we wish to call attention to the following facts as a preliminary to our discussion.

1 The principles advanced by Kahn namely the type of antigen the relative proportions and concentrations of the serum and antigen and the thorough mixture and agitation of the two are adhered to in the micro test.

2 Kline, Littman and Mill have shown that there is an agreement of 95.9 per cent between the micro and macro Kahn tests.

3 Kahn⁷ himself has stated that, providing the principles of thorough mixture and agitation as well as proportionate adjustments of the serum and antigen quantities are adhered to, satisfactory and "highly accurate" micro reactions can be performed with as little as 0.025 and 0.05 c.c. of serum, even quantitative reactions being possible with these small quantities.

Finally, if these premises are not acceptable, then the remarks to follow may be considered as applying to all precipitation tests based upon the principles formulated by Kahn and as drawn from the personal experience of one of us (R. A. K.) with the regular Kahn macro-test, upon our present experiences with the micro-test, and upon the experiences of others with the Kahn macro procedure as reported in the current literature.

The question of which some desire, apparently, to make an issue is: Shall the Kahn test be accepted as an *additional* or an *exclusive* method for the study of syphilis?

We believe that this is a matter to be decided only after a consideration of many factors, that it concerns not only the laboratory and the clinician, the serologist, the syphilographer, and the practitioner at large, but not least of all, the *patient*! We believe, further, that no discussion of this subject can neglect or pass over as irrelevant a consideration of certain basic facts of essential importance.

First of all, it must be taken into consideration that reports of serologic investigations are made, not only to serologists and syphilographers, but to physicians at large and none can deny that *it is not the test but its clinical interpretation* which is of paramount importance.

None can deny, also, that in no small proportion of cases the serologic examination comprises the major portion of the examination for evidence of syphilis, that many physicians exercise but little curiosity as to the delicacy and reliability of the method whereby it is performed, or the training, skill, or experience of the serologist by whom it is performed, and that for all too many, a positive reaction—however obtained—suffices as indisputable evidence of syphilis and a negative reaction as evidence of its absence or cure.

These are facts common to the knowledge of every serologist and syphilographer and recognized by many physicians, why else the discussions of the "menace of the weakly positive Wassermann reaction" and the clinical pleas for an infallible serologic test?

Again, it must be appreciated, as has been emphasized so many times by so many writers, that serologic examinations are not tests for syphilis but tests for evidences of *reaction to syphilis*, that their occurrence depends, not upon the fact that the patient has syphilis, but upon the reaction of his tissues to the infection. Hence, where there is but minimal tissue reaction—as in latent or dormant syphilis—there can be no serologic reaction. An infallible serologic test, therefore, is impossible by the nature of the situation—there is no procedure with which false negative reactions cannot be obtained.

Furthermore, neither the complement-fixation nor the precipitation tests are procedures dependent upon the presence of true or biologically immune

bodies, both are reactions dependent upon variations in the physical characteristics of the serum—markedly constant in syphilis, it is true—but physico colloidal rather than immune in character

Finally the skill of the performer may definitely influence the results of the reaction to be reported to and interpreted by the clinician

The arguments advanced to support the exclusive use of the Kahn test in the diagnosis and study of syphilis are based upon the following major premises

1 *Simplicity of Technique*—A perusal of the literature by one not a serologist, would lead to the impression that the technique of the Kahn test was of such surpassing simplicity that its satisfactory accomplishment could be acquired by any one within a very short time

Granting the small amount of apparatus and the few reagents required the test, nevertheless has its own inherent complexities not the least of which is the *preparation and titration of a satisfactory antigen the constant accurate adjustment of the serum and antigen proportions for the test and especially the proper reading and interpretation of the results*. It is in the border line case without clear cut clinical evidence in which the clinical evidence is least conclusive that serologic tests should furnish the utmost in the way of evidence, and it is in the border line case the weakly reacting case that the Kahn reactions are the most difficult to read and interpret. Upon this point nearly all observers are in entire accord

We do not believe that the Kahn test is so simple and easy that any one, no matter what their previous training can forthwith perform it accurately and reliably. The diffusion of such an impression is unwise unfortunate untrue, and fraught with peril for the patient

We grant the inherent complexities of the complement fixation test and consider that this very fact should furnish an additional safeguard as tending to restrict the performance of this test to those well grounded and well trained and competent to undertake its performance with understanding and a consciousness of the responsibility involved

Furthermore we believe that it is easier to teach a technician to perform a reliable complement fixation test *the results of which shall be clear cut and capable of being read with a minimum of confusion* than to attain a similar objective with the Kahn test—*this being especially true of weakly positive reactions*

The Kahn test is simple in the minimum of reagents and apparatus required but we do not believe that this simplicity extends to its principles its satisfactory performance, or its accurate interpretation

The Wassermann technique is *not* simple it is true, but the trained serologist is apprised of technical errors by disturbance of the controls or, indeed by the very character of the reactions he obtains safeguards not applicable to the Kahn test

2 *Minimal Time and Labor Required*—An extraordinary emphasis has been laid upon the fact that the time and labor involved in the performance of Kahn tests are much less than required for the performance of the comple

TABLE II
COMPARISON OF KAHN AND WASSERMANN TESTS IN SYPHILIS UNDER TREATMENT

NO	PT	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	REMARKS
1	B J	4	44400	4	44400	0	44400	0	44000	±	44000	0	44000	Treated
2	W G	0	Antic	0	44000	0	44400	0	44100	0	44400	0	44400	"
3	W B	0	40000	1	43000	0	44400	0	44100	0	44400	0	44400	"
4	S	3	Antic	±	0	3	44400	±	44400	±	44400	±	44400	"
5	P	0	44410	1	44400	±	44440	±	44440	2	44000	2	44000	"
6	Pe	0	44000	±	44000	2	22200	2	22200	2	44000	2	44000	"
7	M C	4	44440	0	33330	0	44000	0	44000	0	44000	0	44000	"
8	W J	1	0	±	0	0	0	0	0	0	0	0	0	"
9	H	0	10000	0	44400	0	44220	0	44220	0	44220	0	44220	"
10	Ha	4	44444	3	44444	2	44400	4	44444	4	44444	4	44444	"
11	E	±	44444	2	44440	4	44410	0	40000	0	40000	0	40000	"
12	Hu	1	44400	0	44432	0	44100	0	44000	0	44000	0	44000	"
13	F	0	44444	3	44444	2	44444	4	44441	4	44441	4	44441	"
14	C	1	44400	0	44400	4	44440	0	41000	0	41000	0	41000	"
15	W	2	44400	0	44400	0	44000	0	0	0	0	0	0	"
16	Pe	2	0	2	44000	0	0	0	0	0	0	0	0	"
17	N	0	Antic	0	0	0	0	0	0	0	0	0	0	"
18	X	0	0	±	44000	0	0	0	0	0	0	0	0	"
19	Dv	0	0	0	44400	0	40000	2	0	2	0	2	0	"
20	Wa	4	44441	4	44430	4	44200	1	44000	1	44000	1	44000	"

TABLE II—CONT'D

NO	PT	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	KAHN	WASSER MANN	REMARKS
21	Ca	0	10000	0	0	0	0	0	0	0	40000	0	40000	Primary lesion
22	R S	0	0	0	0	0	0	0	0	0	0	0	0	"
23	A	4	4440	3	4440	0	0	0	0	0	0	0	0	"
24	J S	1	Antic	3	44000	0	0	0	0	0	0	0	0	"
25	Y J	0	40000	0	21000	0	0	0	0	0	0	0	0	"
26	V B	3	0	4	44400	4	4440	0	0	0	0	0	0	"
27	H I	1	44000	0	41000	0	0	0	0	0	0	0	0	"
28	J H	1	0	1	0	0	0	0	0	0	0	0	0	"
29	Wb	0	40000	0	44400	0	0	0	0	0	0	0	0	"
30	A G	0	0	0	0	1	40000	0	0	0	0	0	0	"
31	B H	3	44400	4	44400	0	0	0	0	0	0	0	0	"
32	B C	4	44400	±	40000	3	4444	4	4440	4	4440	4	4440	"
33	C V	2	0	2	4444	0	4444	0	0	0	0	0	0	"
34	J A	0	44400	0	44400	±	20000	0	20000	0	20000	0	20000	"
35	R G	0	0	0	11000	0	0	0	0	0	0	0	0	"
36	L B	1	44400	0	0	0	0	0	0	1	44400	1	44400	Primary lesion
37	G B	0	44400	0	44400	2	44000	0	44000	0	44000	0	44000	"
38	M	0	0	0	0	4	44400	0	44400	0	44400	0	44400	"
39	T	2	40000	0	0	0	0	0	0	0	0	0	0	Treated
40	R E	0	44000	±	Antic	±	Antic	±	Antic	±	Antic	±	Antic	"

ment-fixation test and this is advanced as a powerful argument for the exclusive adoption of the Kahn test

While admitting the fact, we are not inclined to attribute to this argument the paramount importance which has been given to it by others

We are not convinced of the necessity for precipitate haste in the diagnosis, rather, we agree heartily with whoever said "Be quick to suspect syphilis but slow to diagnose it" Syphilis, at best, is a refractory disease and, under the most ideal conditions, its treatment is a tedious procedure So great is the influence of a diagnosis of syphilis upon the future of the patient, so important its relation to his life, his family, his friends, and even his every day existence that we are anxious to be sure rather than precipitate in our diagnosis We do not regret nor grudge whatever may be necessary in time or labor to render the diagnosis relatively free from error or to safeguard the efficient treatment of this dreaded malady We are quite confident that with our own serum under examination, time and labor expended in its study are as chaff before the wind and we are willing to grant the patient no less consideration

"The clinician or internist cannot evade a thorough physical examination because it is time consuming nor the surgeon the adoption of an efficient technic simply because it is laborious The serologist and syphilographer in a similar situation must take the same stand"

3 *Delicacy and Specificity*—The essential requisite for a satisfactory serologic procedure in syphilis is relative specificity, relative freedom from the occurrence of false positive reactions The greater the degree to which this attribute can be combined with delicacy, the nearer the approach to the ideal

Investigations concerning the specificity of the Kahn test are, of necessity, largely based upon a comparison of this procedure with the Wassermann test and the fact well known to serologists but often not equally as well appreciated by physicians at large, that, unless technical details are described the phrase "Wassermann test" conveys no idea of the reliability of the method employed, suffices to explain the varying results reported of agreement and disagreement between the Kahn and Wassermann tests

Granting, however, a delicate, and relatively specific method of complement-fixation, it may be accepted as demonstrated that the Kahn test will agree with the complement-fixation test in from 90 to 95 per cent of cases, the figures depending upon the skill with which both procedures are applied and the extent of the series studied

It has also been demonstrated, however, that false negative reactions may be obtained with *either* test, with the Wassermann test because of the influence of natural amboceptor in the tested serum, and also of substances whose nature is unknown but which are capable of producing results similar to those caused by an excess of amboceptor,^{2 10, 11 12} with the Kahn test for reasons not entirely explained, and with *both* tests for reasons inherent in the disease and the reaction to the disease on the part of the patient

These false negative reactions occur, *not only in dormant, but also in active syphilis and constitute therefore, a very definite source of error when only one test and only one examination is taken at its face value*—as is the regrettable but too common tendency of many physicians

Very fortunately however, such false negative reactions while occurring with both tests, not infrequently do not occur simultaneously so that one test may be positive and the other negative. We consider this an extremely valuable circumstance as supplying an additional safeguard and *furnishing a potent argument for the coincident use of both procedures*

Incidentally, while the complement fixation reaction and the precipitation reaction have much in common in the proffered explanations of their mechanisms, the fact that one may be positive and the other negative on the same serum at the same time suggests a difference either in the mechanism or the substances involved

While the occurrence of false negative reactions is of importance in relation to the serodiagnosis of syphilis it is of still more importance and especially when misinterpreted when serologic procedures are utilized as a guide or control of treatment

Large series of comparative Kahn and Wassermann tests may show a high incidence of agreement and yet the gross figures may fail to bring out facts seen by a comparison of the two tests on the same patient at different times and under varying circumstances

We present a series of 40 cases all syphilitics under treatment, upon whom from two to five examinations were made at varying intervals

We call attention first to the constancy of the Kolmer test and its graphic quantitative character secondly to the lack of consistent quantitative comparison of the micro Kahn with this method and finally to the consistent false negative reactions obtained with Cases 2 3 9 12 16 18 19, 22 24, 29, 34, 35 and 36

Also note the cases giving doubtful Kahn reactions and clear cut positive Wassermann reactions and those in which the Kahn test is neither positive earlier nor persists longer than the Wassermann and we do not find therefore that the Kahn test is *always* as has been stated positive sooner or remains positive longer than the Wassermann in syphilis under treatment

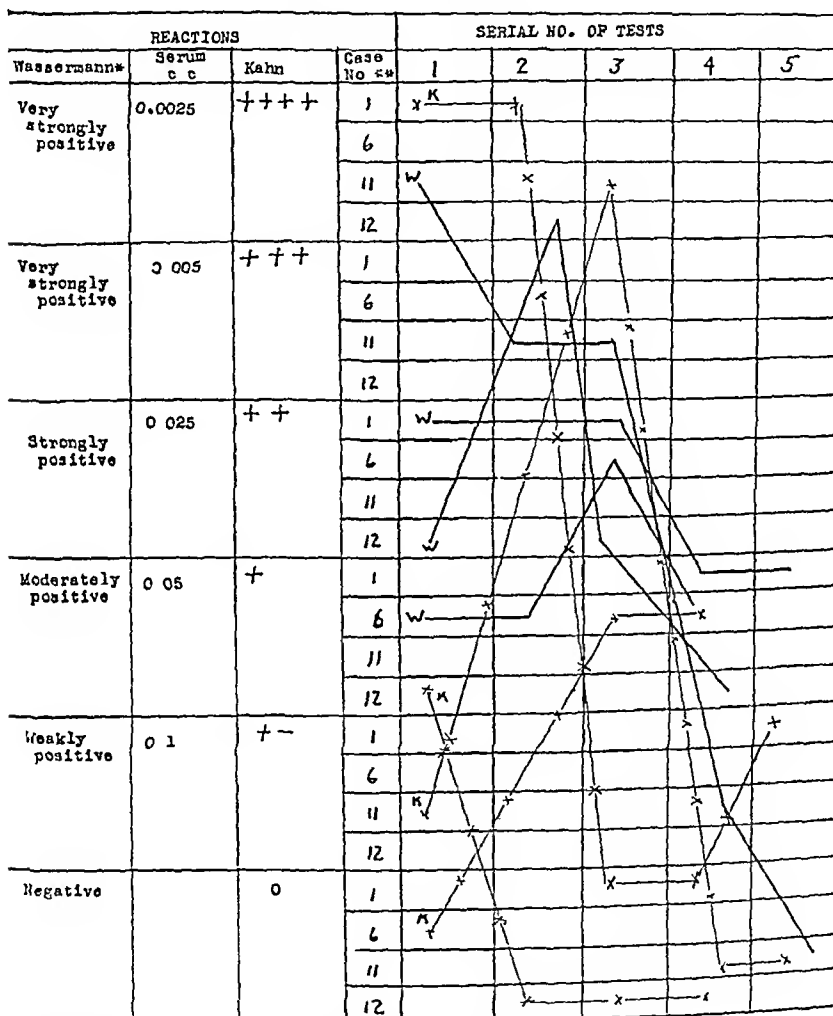
If these differences are presented in graphic form as in Chart I, they are even more strikingly apparent

From the numerous comparisons which have been made of the Kahn and Wassermann tests the latter comprising a variety of methods including that described by Kohner which is conceded to be of exceeding delicacy and possessing a high degree of relative specificity we believe that the Kahn test has been shown to be possessed of a high degree of relative specificity and delicacy. It has also been shown however that with either test false negative reactions may be obtained and what is of still greater importance the occurrence of false positive reactions has been demonstrated with the Kahn test in a varying number of cases their incidence we believe, being between 3 and 5 per cent

This fact alone constitutes a most potent argument against the *exclusive* use of the Kahn test for the serodiagnosis of syphilis *as long as the acceptance and clinical interpretation of serologic reactions in syphilis is based upon their face value, as is true in no inconsiderable number of cases*

The next most important single reason against the adoption of the Kahn test as an exclusive method is the fact that, when the Kolmer and Kahn tests (either micro- or macro-methods) are done routinely, variations occur in from 4 to 5 per cent of sera of individuals clinically regarded as syphilitic, and especially of patients under antisyphilitic treatment, these variations con

CHART I
GRAPHIC CHART OF DIFFERENCE BETWEEN KAHN AND WASSERMANN TESTS
IN TREATED SYPHILIS



*Wassermann reaction recorded in smallest reacting serum dose.

**Refers to cases in preceding table

Wassermann

Kahn

isting of negative reactions with one test while the other is coincidently positive

4 A great deal of emphasis has been laid on the fact that the Kahn test has been adopted as an exclusive test by the Michigan State Department of Health Laboratories and by the United States Navy

As previously said in another place¹³ The fact that the originator of the test is Immunologist of the Michigan State Department of Health Laboratories may be regarded as a factor of some importance in its exclusive adoption by these laboratories. The difficulties involved in the satisfactory performance of Wassermann tests aboard ship or in isolated Naval Stations, and the fact that all Naval Surgeons are not necessarily accomplished serologists¹⁴ has certainly not been without influence on the adoption of this test by the Navy to the exclusion of the Wassermann. We do not find in these facts any obligation upon the serologic fraternity at large forthwith to do likewise.

We regard as unassailable the assumptions that, in all that has to do with the study of syphilis, the patient is intimately concerned, that, both as to diagnosis and the initiation, continuation, cessation, or resumption of treatment, every possible safeguard should be employed and zealously sought for, that, so great and disastrous and practically impossible ever to eradicate is the stigmatizing aftermath of a false positive diagnosis, and so wide spread the calamitous potentialities of a premature cessation of treatment that every possible means, *no matter how laborious or time consuming*, should be employed to prevent these errors.

We deplore a blind and unquestioning acceptance of any serologic procedure as constituting of itself a thorough, complete or infallible examination for evidence of syphilis. We deny the suitability of the Kahn or any other precipitation test for this purpose, and we believe that its true value and proper place is as an additional serologic method for the diagnosis and study of syphilis to be used *with and not in place of* the complement fixation test.

If still another serologic procedure of comparable value to the Kahn and Wassermann tests should come to light, we would rather add it to our armamentarium than discard either.

SUMMARY

A report of 2116 comparative micro Kahn and Kolmer tests is presented as a result of which it is concluded that the micro Kahn test is a satisfactory additional method for the serologic study of syphilis.

The suitability of the Kahn test as an exclusive method for the serologic study of syphilis is discussed and the conclusion reached that it should be used *in conjunction with and not in place of the Wassermann test*.

REFERENCES

- ¹Kahn, R. L. A Simple Quantitative Precipitation Reaction for Syphilis, Arch. Dermat and Syph., 1922, v, 570
- Kahn R. L. Serum Diagnosis of Syphilis by Precipitation, 1925. Williams and Wilkins Co. Baltimore Md.
- ³Kline, B. S. and Young A. M. A Microscopic Slide Precipitation Test for Syphilis, Jour Am Med Assn., 1926, lxxxvi, 928
- ⁴Kline B. S., Littman S. and Mill A. B. Results of 2600 Microscopic Slide Precipitation Tests for Syphilis with Clinical and Serological Comparison, Am Jour Syph., 1926, x, No 4, p 636
- ⁵Kolmer J. A. A New Complement Fixation Test for Syphilis Based upon the Results of Studies in the Standardization of Technique Am Jour Syph., 1922 vi 82
- ⁶Kahn, R. L. Serum Diagnosis of Syphilis by Precipitation. Williams & Wilkins Co., Baltimore, Md., p 147
- ⁷Kahn R. L. Micro Kahn Reactions, Jour Am Med Assn., 1926, lxxxvi, 2092
- ⁸Kilduffe B. A. The Standardized Wassermann Test. JOUR. LAB AND CLIN MED., 1924, ix, No 12, 868

- ⁹Kilduffe, R A Relation of Natural Antisheep Amboceptor of Human Serums to Kolmer's Quantitative Complement Fixation Test for Syphilis, Arch Dermat and Syph, 1924, 1, 745
- ¹⁰Kilduffe, R A The Influence of the Natural Antisheep Hemolysins of Human Serum upon the Production of Anomalous Reactions in the First Tube of Kolmer's Quantitative Complement Fixation Test for Syphilis, Jour Lab and Clin Med, 1924, 1, No 2, p 93
- ¹¹Detweiler, H K The Practice of Medicine, 1923, p 295
- ¹²Kolmer, J A Infection, Immunity, and Biologic Therapy, ed 3, 1924, W B Saunders Co, p 488
- ¹³Kilduffe, R A Wasserman vs Flocculation Tests in the Serologic Study of Syphilis The Suitability of the Kahn Test as an Office Procedure, Arch Path and Lab Med, 1926, 11, No 6, p 866

THE PREPARATION OF POTASSIUM AND SODIUM TETRABISMUTH TARTRATES*

By PHILIP ADOLPH KOBER,† CHICAGO, ILL

INTRODUCTION

THE value of bismuth therapy in syphilis as shown by Cole, Farmer and Miskdjian¹ depends upon the solubility and speed of absorption of the bismuth preparation used. They found that the bismuth tartrates were the most readily absorbed compounds that they studied, but even these compounds required a long time for absorption when suspended in oils.

The following work was undertaken with a view to making a soluble bismuth preparation of definite chemical composition, which could be administered in aqueous solution, free from the slow absorption, nodule formation and danger to fat embolism, due to the use of fat suspensions. For rapid absorption it seemed desirable to avoid the use of the potassium compounds, owing to their objection found by many workers in pharmacology and medicine² and therefore most of the work was done on sodium compounds.

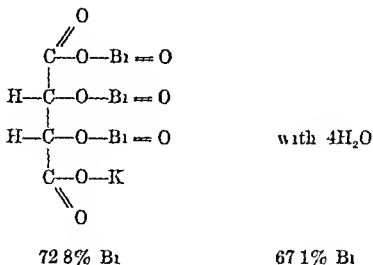
IMPORTANT SOLUBLE BISMUTH COMPOUNDS

Theoretically and actually found, there are many different compounds of bismuth and tartaric acid. Of the water soluble preparations which are probably more effective on account of speedier absorption and greater penetration, all of them also contain in addition to bismuth and tartaric acid, alkali metals, either sodium or potassium or both. Ordinary bismuth salts of tartaric acid have a too great tendency to hydrolyze into insoluble bismuth oxide or basic salts to be desirable for syphilitic treatment. Therefore the more firmly the bismuth is bound to the tartaric acid in the form of a complex, the greater efficacy can be expected as the bismuth must remain in the form of a soluble organic complex long enough to allow absorption from the site of injection and permit distribution throughout the body by means of the blood stream. Chemical tests show that the most firmly bound bismuth compounds heretofore isolated are the so called tribismuth alkali tartrates,

*Read before the Organic and Biological Group American Chemical Society Sectional Meeting Madison Wisconsin May 29 1926

†From the Research Laboratories of G D Searle & Company

of which the only known example is potassium tribismuth tartrate. The following formula has been assigned to this compound



This compound and its preparation were first described by Rosenheim and Vogelsang¹ in 1906, and has been on the market and used clinically both here and abroad. For reasons stated above, a sodium tribismuth tartrate seemed desirable and possibly of value. No method was available in the literature which described the isolation of this compound. Rosenheim and Vogelsang attempted to isolate the sodium compound but they stated it would not crystallize out similarly to the potassium compound. Klauder⁴ makes the statement that the sodium salt is unstable which indicates in the light of the work reported here that the sodium compound had not been successfully isolated. It is probable that Rosenheim and Vogelsang in their experiments, and possibly others too, actually had some of the sodium tribismuth tartrate compound present in their solutions together with an excess of reagents, but in this form its usefulness for syphilitic treatment could not and never did become practical.

ALKALI TETRABISMUTH TARTRATES

On considering the method of Rosenheim and Vogelsang which consisted in digesting bismuth subnitrate with tartaric acid and an excess of alkali while heating, I came to the conclusion that from thermodynamic reasoning the reaction ought to be conducted in the cold or at least without heating. Furthermore, since the nitrate group does not enter into the composition of the compound desired, its presence may interfere with the reaction. For these reasons the reaction was tried with a slight excess of sodium hydroxide in the cold, using bismuth hydroxide as a source of bismuth and shaking with a mechanical shaker. The bismuth hydroxide at first dissolved fairly rapidly, but soon it dissolved more and more slowly. A number of experiments, however, showed that the longer I shook the mixture, the more bismuth hydroxide dissolved. At the end of about 144 hours (6 days) the mixture seemed to come to an equilibrium and no more bismuth hydroxide seemed to dissolve. On filtering I had in solution of course, a sodium bismuth tartrate. A few experiments with small portions of a filtrate soon showed that a half volume of 95 per cent alcohol gave me a copious yield of

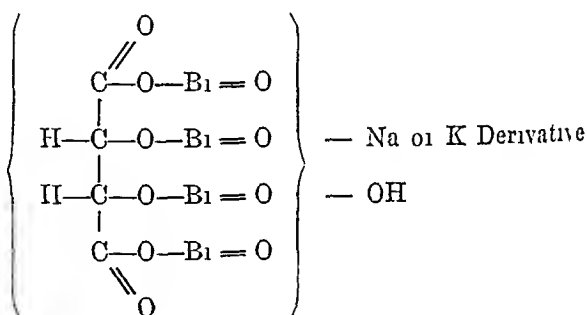
precipitate The precipitate after washing with 50 per cent alcohol several times to remove the mother liquor and finally with 95 per cent alcohol and drying in the air at room temperature gave a water soluble powder containing 72.9 per cent of bismuth and 4.6 per cent of water of hydration, or for an anhydrous substance 76.4 per cent of bismuth. Since the anhydrous sodium tetrabismuth tartrate could not contain more than 74.2 per cent of bismuth, it was evident that this preparation contained more than three molecules of bismuth. The theoretic per cent for bismuth and water for sodium tetrabismuth tartrate is seen in Table I.

TABLE I

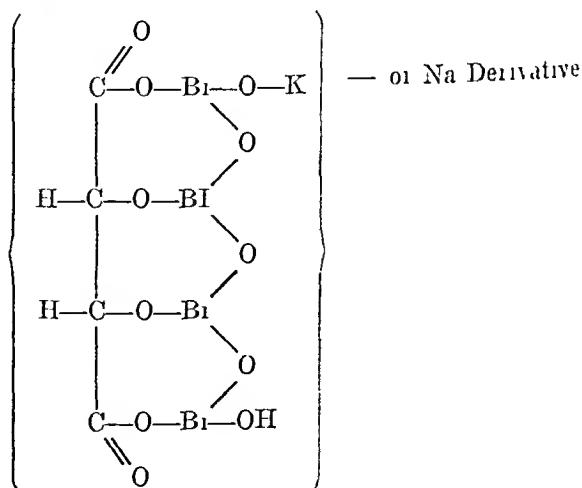
	ANHYDROUS		1H ₂ O		2H ₂ O		3H ₂ O	
	Bi%	H ₂ O%	Bi%	H ₂ O%	Bi%	H ₂ O%	Bi%	H ₂ O%
Theory for Bismuth Tartrate	74.2	2.09	72.7	7.12	71.2	6.01	69.6	
Found	76.4					4.60	72.9	
Theory for Tetrabismuth Tartrate	77.1	1.63	75.7	7.12	71.2	4.73	73.2	

From these data I was forced to the conclusion that this sodium tartrate compound was sodium tetrabismuth tartrate and I have tentatively assigned the following formula to it:

Mol. Wt.—1086
77.1% Bi
Formula I



Mol. Wt.—1102
75.8% Bi
Formula II



*The bismuth content was obtained by dissolving 0.500 gm. of the compound in 25 c.c. of water heating the solution to 50° C. and adding 1.0 gm. of sodium hydrosulphite (Na₂S₂O₄) dissolved in 5 c.c. of 10 per cent of ammonia then filtering washing drying and weighing the metallic bismuth.

If these findings and conclusions with the sodium compound were correct, then it ought to be possible to get a potassium compound of tartaric acid with even more bismuth content than any found on the market, since most of them approximate in bismuth content that of a tribismuth tartrate.

On substituting potassium hydroxide for sodium hydroxide in the method developed for the sodium compound I had no difficulty in getting a fair yield on the first trial using otherwise the exact technique I did for the sodium process. Perhaps by using more suitable proportions of alcohol since the solubilities of the two substances undoubtedly differ, a greater yield would have been obtained. The analytic data however agree with the theory of a tetrabismuth tartrate even more closely than did the sodium compound.

TABLE II

	ANHYDROUS		H ₂ O		2H ₂ O	
	Bi%	H ₂ O%	Bi%	H ₂ O%	Bi%	H ₂ O%
Theory for Tribismuth Tart	72.9	2.0	7.14	4.02	70.0	7.0
Found	75.8			2.76	73.8	
Theory for Tetrabismuth Tart	75.8			3.16	73.3	

PROPERTIES OF TETRABISMUTH TARTRATES

These tetrabismuth tartrates are finely divided powders, odorless and tasteless, permanent in the air at ordinary room temperatures. At higher temperatures, they seem to suffer a change probably absorbing carbon dioxide and other acid vapors from the air so that they require additional alkali before they will dissolve in water. On contact with water these preparations first form a gel and then dissolve. Accurate solubility determinations have not yet been made, indications are that the solubility is somewhere near 40 per cent, one part of water at room temperature does not dissolve quite one part of these tetrabismuth tartrates whereas one and a half parts usually will.

Solutions of these tetrabismuth tartrates are alkaline in harmony with the formula and so far as we know are perfectly stable. The alkalinity can be decreased by titrating with N/10 sulphuric acid using phenolphthalein as an indicator to a hydrogen ion concentration of about 8.486 at which point the solutions up to 10 per cent are still stable indefinitely. Heated to 70° C for three fourths hour the solution seems to be unchanged, while heating for five minutes at 100° produced only a very slight cloud and a slight increase in alkalinity but which on cooling slowly but practically disappeared.

The alkalinity can be also decreased by absorption of iodine, with the formation of colorless solutions of iodides and iodates of the tetrabismuth tartrates, which also seem stable at room temperatures.

On adding a gram equivalent of acid to solutions of tetrabismuth tartrates a precipitate is produced, which redissolves in an equivalent amount of alkali. This base is very gelatinous and seems to be close to a permanent suspension.

These tetrabismuth tartrates are not precipitated by blood proteins, carbohydrates, etc

TOXICITY

Raiziss, Seveiac and Windicov⁵ quote Sazeriac and Levaditi, who were the first to study bismuth tartrates as curative agents for syphilis, as stating that their sodium and potassium bismuth tartrate killed white rats when 5 mg per kg was injected intravenously. Raiziss and associates found their potassium tribismuth tartrate, a supposedly tribismuth tartrate, to be tolerated up to 10 mg per kg, but killing at 15 mg per kg. Our preliminary results with white rats indicate that these tetrabismuth tartrates are considerably less toxic being tolerated when injected intravenously up to about 25 mg per kg. Injected intramuscularly these tetrabismuth tartrates are tolerated in something over 600 mg per kg.

PREPARATION OF TETRABISMUTH TARTRATES

Two hundred grams of bismuth subnitrate were dissolved in 270 cc of concentrated nitric acid and made up with water to about 1500 cc. Then with rapid stirring 300 cc of saturated sodium hydroxide (50 per cent) were added to precipitate the bismuth hydroxide in a finely divided condition. The precipitate was then filtered upon a suction filter, washed and resuspended in water, filtered, washed with distilled water three or more times until all of the mother liquor had been removed.

Into a liter bottle or flask were weighed 25.0 gm of tartaric acid and 75 cc of distilled water were added. Then 28 cc of saturated sodium hydroxide (50 per cent) were added with stirring and cooling. When the liquid had cooled at 14-15° C the bismuth hydroxide, prepared as described above, was added and after stoppering the bottle or flask securely, the mixture was shaken on a mechanical shaker for one hour or two, at three hour intervals during the first day. This shaking was repeated two or more times a day, for 6 to 7 days. Longer standing or shaking caused no harm, but the amount of bismuth hydroxide dissolved by the alkaline tartrate solution was hardly increased over the amount dissolved during the first six days. Attempts to shorten the period by heating decreased the amount and purity of the yield.

The mixture at the end of six days was filtered through a porous glass Buchner suction filter, or through a hard filter paper. After washing the undissolved bismuth hydroxide with 50 cc of distilled water, the total filtrate equalled 350 cc.

On adding 175 cc of 95 per cent alcohol to the filtrate with stirring, a copious precipitate was obtained which was filtered on a Buchner funnel with suction, washed and suspended in 100 cc of 50 per cent alcohol, filtered and washed, resuspended, filtered and washed with 95 per cent alcohol three or four times or until the filtrate was neutral to litmus paper. After drying at room temperatures for several days, the yield was in the neighborhood of 125 gm or 89 per cent. On substituting the same proportion of potassium hydroxide for sodium hydroxide, the yield was about 30 per cent. Larger yields could undoubtedly be obtained with the potassium compound if larger amounts or other proportions of alcohol were used.

PURIFICATION OF TETRABISMUTH TARTRATES

It seems possible to purify these tetrabismuth tartrates by dissolving in water and precipitating with alcohol. The following experiment is of interest. Two small yields from some preliminary experiments were obtained, one having 13 gm with a bismuth content of 67 per cent, the other having 65 gm with a bismuth content of 67 per cent. To 300 cc of water together with 5 cc saturated sodium

hydroxide. A little excess of alkali is sometimes advisable to prevent the alcohol from precipitating the tetrabismuth tartrate base, through the interaction of the alcohol and the alkali of the complex. On adding 130 c.c. of 95 per cent alcohol with stirring, filtering and washing with alcohol and drying obtained a yield of 59 gm., with a bismuth content of 73.8 per cent.

The fact that mixtures of alkali bismuth tartrates having a bismuth content equivalent to that of a so-called tribismuth tartrate can by reprecipitation give a large yield of a tetrabismuth, indicates if it does not prove that these so-called tribismuth tartrates are mixtures of tetrabismuth and dibismuth tartrates. Preliminary toxicity results show that the tetrabismuth tartrates are much less toxic than these tribismuth tartrates indicating that the latter contain bismuth or compounds containing bismuth, which easily splits off or ionizes bismuth.

CONCLUSIONS

(1) It is shown that contrary to previous directions alkaline solutions of tartaric acid dissolved bismuth hydroxide more efficiently at a low temperature. (2) When the reaction is allowed to go to completion, tetrabismuth tartrates result, instead of tribismuth tartrates as has been heretofore assumed. (3) Two new compounds sodium tetrabismuth tartrate and potassium tetrabismuth tartrate have been described and the processes given for their preparation. (4) Preliminary results indicate that these new tetrabismuth tartrates are much less toxic than other bismuth tartrates isolated heretofore. (5) That certain data given indicate if they do not prove that the so-called tribismuth tartrates mostly heretofore used in syphilitic therapy, are only mixtures of tetra- and dibismuth tartrates. (6) Preliminary experiments show that the sodium tetrabismuth tartrate can be put up in an aqueous solution and administered intramuscularly.

REFERENCES

- ¹Cole, Farmer, and Miskdjian. *Arch. Dermat. Syph.*, 1926, xiii, 219.
- ²U. S. Dispensatory, ed. 21, 1926, 897.
- ³Rosenheim and Vogelsang. *Ztschr. f. Anorg. Chem.*, 1906, xlviii, 205.
- ⁴Klauder. *Arch. Dermat. Syph.*, 1923, vii, 722.
- ⁵Raiziss, Severac and Windicov. *Am. Jour. Clin. Med.*, August 1923, *Arch. Dermat. Syph.*, 1925, xii, 661-669.

A MONILIA FROM THE RESPIRATORY TRACT*

BY FREDERICK W. SHAW, M.D., RICHMOND, VA

AFFECTIONS of the bronchi and lungs due to *Monilia* Persoon, 1797, are found throughout the tropics, especially in places having a damp climate. The affection may be met with in Europe and in America. Castellani¹ states that in Ceylon the malady is generally due to *Monilia tropicalis* Castellani, 1910, and that the same fungus may be found in cases coming from South India and from the Malay States. In some cases other species of the fungus may be observed, but it is doubtful whether all of these are really pathogenic.

The genus *Monilia* Persoon, 1797, is usually defined¹ as Oosporiaceae possessing in situ budding forms and mycelial threads, which latter are often long and branched, in cultures mostly budding forms, but sometimes filaments, in which thallospores of the blastospore type are formed. Dextrose and often other carbohydrate media fermented with the production of gas.

Much confusion has resulted from the attempts to classify the genus *Monilia*, and Castellani has suggested that the classification should be based, not only on morphologic data, but also on biologic characters and immunization phenomena.

The following case was observed in St. Philip Hospital, Richmond, Va.

REPORT OF CASE

G. B., aged twenty-five years, female, married, colored. Admitted January 11, 1926.

Three months previous to admission she experienced stiffness and soreness from the neck spreading downward to the knees. She had toothache for years. Immediately preceding the present attack, there was severe toothache in the right lower jaw. The right jaw was much swollen.

Physical examination. Patient was a well-developed colored woman of about the stated age. Mouth showed a severe pyorrhea. There were no cardiac murmurs. Some dullness over right front of chest extended downward to the fourth rib. Posteriorly, the dullness extended to near the angle of the scapula. Tubular breathing was heard over the upper portion of the right lung, extending downward to the third rib anteriorly, and below the spine of the scapula posteriorly. Occasional râles on coughing were heard throughout the right lung. Râles were occasionally present in the upper left lung. Tubular breathing at left apex. Pectoriloquy over both apices.

Patient did not have the appearance of being acutely ill. Very little sputum and cough, no chills, no sweats. Appetite was fine, she slept well.

X-ray of chest showed an apparently well-advanced tuberculosis involving the upper right lobe. This had progressed to consolidation from the apex downward to the level of the second rib. The condition did not appear healed.

The temperature on admission was 100° F. It varied from 99° F. to 100.2° F. and became normal on February 11, 1926.

The patient received seven drops of a saturated solution of potassium iodide three times a day, beginning on January 28, 1926, and this was continued until her departure from the hospital, February 20, 1926.

*From the Department of Bacteriology, Medical College of Virginia.
Received for publication February 26, 1927.

THE ORGANISM

Examination of the scanty sputum failed to show the tubercle bacillus, either by microscopic examination or by guinea pig inoculation. The sputum contained small firm white granules which when crushed under a cover glass were found to be composed of thickly matted threads and yeast like bodies.

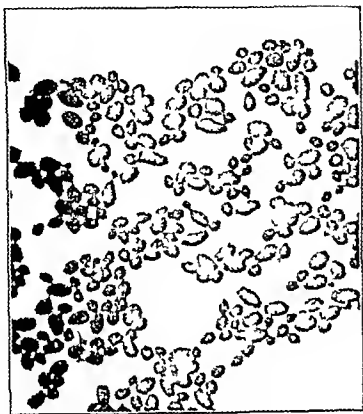


Fig 1—*Monilia richmondii* Gram stain from growth on dextrose agar



Fig 2—*Monilia richmondii* Gram stain from growth in dextrose broth

These granules were planted on maltose agar and the organism isolated. Cultures from the tonsils and teeth were negative for monilia.

The growth on maltose agar which was abundant, was glossy, creamy white with a smooth, raised surface and entire margin. Examination of the growth under the low power of the microscope showed it to be made up of roundish bodies resembling yeast cells. Slide preparations of the growth under the 19 mm objective showed globular yeast-like budding cells, which varied from $14\ \mu$ to $56\ \mu$ (Fig 1).

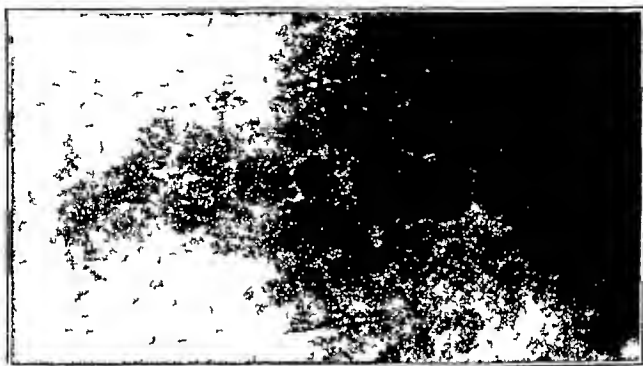


Fig 3—*Monilia richmondii* Moniliform bodies Section of growth in gelatin. Unstained

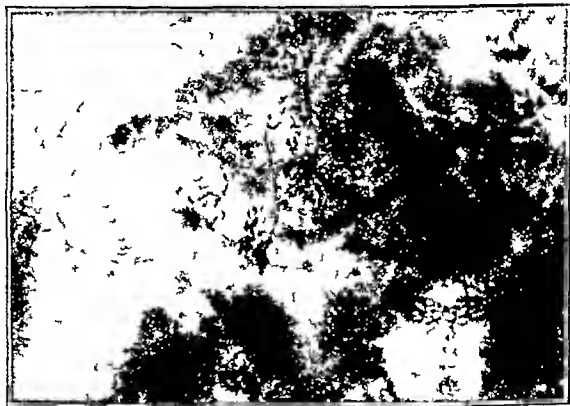


Fig 4—*Monilia richmondii* Conidiophores and conidia Section of growth in gelatin Unstained

Examination of the growth from dextrose broth showed, in addition to the yeast-like cells, long, septate, branching mycelia (Fig 2). Spores were seen on the mycelium at the ends and at the junctions of the septa. The mycelium was from $14\ \mu$ to $18\ \mu$ in diameter, the segments were from $8\frac{1}{2}\ \mu$ to $21\ \mu$ in length, and the mycelial threads were from $47\ \mu$ to $140\ \mu$, or longer.

Milk was rendered alkaline in forty-eight hours without preliminary acidification. There was no coagulation. Acid and gas were formed in dextrose, levulose, maltose, and galactose. The following were not fermented: saccharose, lactose, mannite, dulcitate, raffinose, arabinose, adonite, dextrin, sorbite, muhim. No pellicle was formed on broth or Dunham's solution, and

the medium was clear with the growth at the bottom of the tube. Blood serum was not liquefied.

The organism stained by Gram's method.

No asci were observed.

The growth in a 5 per cent gelatin stab after about a week showed a growth which resembled the root system of a tree. Branched outgrowths occurred from



Fig 5—*Monilia albicans*. Section of growth in gelatin. Untained.

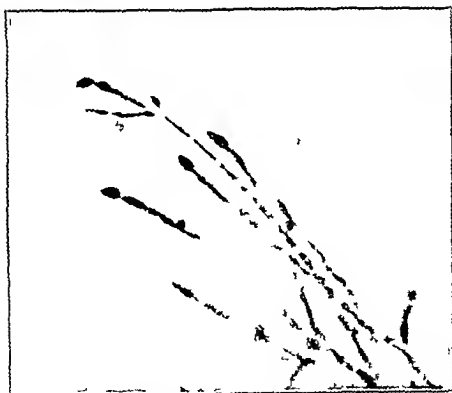


Fig 6—*Monilia richmondii*. Rabbit kidney showing the organism. Stained with dilute fuchsin and decolorized rapidly with alcohol.

the line of stab. These branching outgrowths, when examined with a hand lens, appeared to be made up of small bead like bodies somewhat akin to the growth of *Monilia albicans*. When the gelatin culture was hardened with 10 per cent formalin and thin sections were made, the growth was seen to be made up of moniliform bodies (Fig 3) and structures which resembled conidiophores. The

conidiophores were short and bore conidia which were oval in shape and measured $1\ \mu$ by $3\ \mu$. Branching of the conidiophores was common (Fig 4). The production of this type of conidiophore and conidia was not observed in gelatin stab of *Monilia albicans* (type species, American Type Culture Collection). The character of the formation of the moniliform bodies in *M. albicans* is shown in Fig 5. The articles of the moniliform bodies are always round.

In the preliminary report of this organism³ it was stated that gelatin was not liquefied. This statement was made as the result of using 10 per cent gelatin. By the use of 3 per cent gelatin and employing the single stab method, or by the use of 5 per cent gelatin and a very heavy inoculation, it was found that liquefaction would begin in about three weeks at room temperature.

ANIMAL EXPERIMENTS

Inoculations into the peritoneal cavities of rabbits or guinea pigs produced no lesions. When injected into the circulation of the rabbit there developed, about the fifth day, tetanoid convulsions of short duration followed by the death of the animal. Necropsy showed the kidneys enlarged and studded with very small, whitish granules, the liver, stomach wall, and omentum contained similar granules. The organism was recovered in pure culture from a number of the lesions. The guinea pigs received injections of the culture intracardially. Convulsions with paralysis of the hind legs developed on the fifth day, death followed in a few hours. Necropsy showed that the same pathologic changes had occurred in the rabbit.

Stained histologic sections of the rabbit's kidney showed the whitish granules to be due to accumulations of the fungus (Fig 6).

Intrapulmonary injection into the rabbit produced a caseation and necrosis of the lung at the site of injection, later it produced a general septicemia with lesions similar to those described.

DISCUSSION

The monilia described in this paper differs from *Monilia albicans* in that it does not form a honey-comb growth on dextrose agar, does not coagulate milk, and presents a very different appearance during the development of the moniliform bodies. It differs more markedly from *Monilia psilosis*,⁴ which in gelatin shows a characteristic pine tree growth with fine hair like lateral shoots extending from the entire length of the stab. On dextrose agar, *M. psilosis* produces a rough, yellow growth in contrast to the glossy, creamy white appearance of the organism here presented.

The specific name of *richmondi* has been suggested for this monilia.¹

Thanks are extended to Dr Thomas P. Haslam, who supplied me with the sputum and the case history.

REFERENCES

- ¹Castellani and Chalmers. Manual of Tropical Medicine, 1919, New York, Wm Wood & Co
- ²Boggs and Pincoff. Bull Johns Hopkins Hosp, 1915, xxvii, 407
- ³Shaw. Science, 1926, lxi, 300
- ⁴Ashford. Am Jour Med Sc, 1917, cliv, 157

THE ORIGIN AND NATURE OF THE WASSERMANN ANTIGEN

BY L. G. HADJOPOULOS, M.D. AND REGINALD BURBANK, M.D. NEW YORK CITY

IN THE class of antigenic substances the Wassermann antigen holds a unique place. Contrary to the supposed protein nature of antigenic bodies, the Wassermann antigen, the only known exception is pure tissue lipin. It is capable of fixing complement in the presence of its specific antibody, the luetic reagent, though its parenteral introduction does not give rise to specific antibody production.

Subsequent to the studies of Forssman¹ certain specially prepared tissue extractives were taken as exhibiting true antigenic properties. Later, difficulties in producing a protein free lipin cast doubt on the integrity of similar observations, and tended to prove that all antigens are of protein nature.

During the last year, interest in the subject was revived on the Continent. Doerr and Hallauer,² by the addition of protein radical were able to change Forssman's lipid into a full antigen. After trying various proteins such as pig's serum, internal organs and even hemolyzed erythrocytes of rabbits they came to a belief in the real chemical combination of the lipin protein.

A variant view was taken by Sachs and Klopstock.³ In their opinion lipins are true antigens, but their functions as such are masked in the presence of body proteins and reappear only after the addition of the foreign protein employed, e.g., hog's serum.

By injecting *Spirocheta pallida* into rabbits, Klopstock⁴ claims to have produced antibodies with a better selectivity for spirochete lipins in contrast to organ lipins. He concludes that the serologic changes in syphilis are induced directly by the spirochetes and indirectly through the production of lipotropic tissue antibodies in response to the specific spirochete reaction in the system.

Brandt, Guth and Muller⁵ repeated some of the above experiments and obtained only very weak fixations with the best antigens they had at the time. In a later work they found indications of organ specificity, especially with lipins extracted from brain tissue.

Much⁶ who was one of the early propounders of the true antigenic nature of lipins, in a recent work admits that only certain fats can act as antigens and then only in combination with proteins. A short time ago he alone claimed antigenic properties for lipins; he now is in favor of rejecting the antigenic property of true proteins.

While this controversy was gradually becoming acute in Europe, it occurred to us that the trend of these apparently conflicting opinions was approaching a stage that allowed a better and more intelligent explanation as to the nature of antigens in general and the Wassermann antigen in particular.

From the Department of Laboratories of Beth Israel Hospital, New York City.
Received for publication March 3, 1917.

Since chemically pure lipins are not antigenic but can be rendered so by the addition of some protein radical, whether it be pig's serum, hemolyzed erythrocytes, spirochetes or internal organs, it stands to reason that, in view of the undoubted antigenic nature of protein in general and the luetic reagen fixing property of organ lipins in particular, the something which exhibits two serologically distinct properties (antibody-inducing and antibody fixing) can no longer be considered as a single homogeneous body

We took this view as a working basis in conformity with the above experimental facts, in fractioning an antigen it was only natural to ascribe to the protein fraction the immunity-exciting, *immunogenic* properties and to the lipin fraction the immune body-fixing, *immunophilic* properties. Fractioning of the antigen consisted in separating the two elements and obtaining them in the purest state possible. This was done as follows

TECHNIC OF SPLITTING THE ANTIGEN INTO ITS IMMUNOGENIC AND IMMUNOPHILIC FRACTIONS

As it is essential to have the lipin fraction protein-free, dried and thoroughly pulverized heart muscle tissue was extracted for a week in absolute alcohol at incubator temperature, with a few daily shakings. Part of the clear supernatant fluid was removed and centrifugalized at high speed for fifteen minutes to rid of all traces of protein material held in suspension. The product thus obtained, kept at room temperature for a week, gave a white flaky-granular sediment, a mixture of fats and soaps sedimented by centrifugalization. The final product composed of fatty acids, cholesterol and lecithids represented the *immunophilic* fraction, the regular Wassermann antigen.

To obtain the immunogenic lipin-free protein fraction, a small portion from the top layer of the tissue sediment already once extracted with alcohol, was removed by means of a pipette and washed repeatedly with warm absolute alcohol until the washings were free from all traces of lipins. This was determined by pouring the entire final washing in water. In the total absence of even a trace of opalescence the extraction was considered complete. Nevertheless a further washing in equal parts of alcohol-ether mixture was given to insure the purity of the lipin-free fraction. The sediment thus obtained was dried, pulverized, and a portion of it emulsified in saline, was used for intravenous injection. Table I gives the experimental data showing the correctness of our hypothesis.

Comments—The complement fixations were performed with all three antigens, viz., the whole heart tissue, the delipinized heart tissue (the same material that was used to immunize the animals), and the pure lipin extractive (the antigen ordinarily used for the Wassermann reaction). All three antigens were previously titrated for their antigenic, anticomplementary and hemolytic values. In the course of fixation a marked degree of hemolytic property was exhibited by the serum of the experimentally injected rabbits. This was, however, properly controlled in the standardization of the hemolytic system.

As will be seen in Table I, the delipinized heart tissue was purely im

TABLE I
THE PRODUCTION OF LIFOTROPIC ANTIBODIES IN RABBITS BY THE PARENTERAL INTRODUCTION OF WHOLE AND DELIPINIZED OX HEART TISSUES

INJECTION AND COMPLEMENT FIXATION TEST DATES	COMPLEMENT FIXATION TESTS WITH ALL THREE ANTIGENS									
	WHOLE HEART	DELIP HEART	WHOLE HEART	DELIP HEART	WHOLE HEART	DELIP HEART	WHOLE HEART	DELIP HEART	WHOLE HEART	DELIP HEART
	ANTIG	ANTIG	ANTIG	ANTIG	ANTIG	ANTIG	ANTIG	ANTIG	ANTIG	ANTIG
8/18/25 Preliminary	-	-	-	-	-	-	-	-	-	-
8/18/25 Fixations	-	-	-	-	-	-	-	-	-	-
8/23/25 1st Injection	-	-	-	-	-	-	-	-	-	-
8/23/25 2nd "	-	-	-	-	-	-	-	-	-	-
8/25/25 3rd "	-	-	-	-	-	-	-	-	-	-
8/27/25 4th "	-	-	-	-	-	-	-	-	-	-
8/29/25 1st Fixation	-	-	-	-	-	-	-	-	-	-
9/5/25 2nd "	-	-	-	-	-	-	-	-	-	-
9/11/25 3rd "	-	-	-	-	-	-	-	-	-	-
9/19/25 4th "	-	-	-	-	-	-	-	-	-	-
9/23/25 5th "	-	-	-	-	-	-	-	-	-	-

Experimental Animals Injected with Delipinized Tissue

Whole Heart Tissue

+

++

+++

+

++

+++

+

++

+++

+

++

+++

+

++

+++

+

++

+++

+

TABLE II

INTENSITY AND PERCENTAGE DISTRIBUTION OF NONSPECIFIC WASSERMANN REACTIONS IN
RELATION TO DISEASE

CLINICAL CONDITIONS GIVING RISE TO NONSPECIFIC REACTIONS	INTENSITY OF REACTION	GROUP PERCENTAGES
Septicemias, including Strept Endocarditis, 15 per cent	+, ++	27 per cent
Cardiacs and cardionephritics	+	16 per cent
Anemias and Leucemias	++, +++	8 per cent
Gastric and duodenal ulcers	++	6 per cent
Malaria and lead poisoning	++	4 per cent
Pregnancy, normal	+	61 per cent pathologic conditions
Sundry, normal cases	+	2 per cent
		37 per cent
		39 per cent physiologic conditions

TABLE III

THE DISTRIBUTION OF NONSPECIFIC FIXATIONS IN FORTY SEVEN CASES OF
SUNDY CARDIOPATHIES

CARDIAC PATHOLOGY	NUMBER OF CASES		TOTAL	PERCENTAGE POSITIVES
	POSITIVE	NEGATIVE		
Myocardiacs	4	10	14	28 per cent
Endocardiacs	9	24	33	28 per cent
Totals	13	34	47	28 per cent

munogenic and not immunophilic, i.e., it could excite the formation of specific antibody but failed to fix complement in the latter's presence. Thus a new light was shed upon the subject of antigens. Other facts of secondary importance were that the whole (nonfractionated) tissue was superior for fixation to the delipinized and pure lipin antigens. Although it seemed reasonable to ascribe this to the presence in the whole tissue of both antigenic fractions, we were inclined to think that the slight immunogenic inferiority of the delipinized antigen was not due so much to the lack of lipins in the protein molecule as to the possible chemical alteration brought about by the lipin solvents used. We have definite reasons for believing that ether alters more than alcohol. Lastly, the appearance of the reaction was more gradual than its disappearance. It took about two weeks for the reaction to appear and a little longer to disappear altogether.

ORIGIN OF THE WASSERMANN ANTIGEN

We have already mentioned the observations of Brandt, Guth, and Muller as to the organ specificity exhibited by lipins and especially by those lipins extracted from brain tissue. The organ used in our experiments was ox heart. Consequently, if there is an organ specificity, lipins derived from other sources should at least not react with the same intensity to the serum of our experimental animals. In this respect our work, being but fragmentary, was not incorporated in Table I. Nevertheless, the conclusions drawn from the scant material at hand are rather in favor of such an assumption.

Landau and Held² observed that in thirty cases of endocarditis lenta the Wassermann reaction was positive in ten (30 per cent) and in at least

eight of these there was no other ground for suspecting specific infection. It is the opinion of the above authors that the positive reactions in these cases should be considered as incidental and secondary phenomena of a "disturbed colloidal or lipid balance" in their serums.

It has been our experience for the last ten years that if active serum is used for the Wassermann reaction and a heart tissue plain alcoholic extract for antigen, a certain, although very small percentage (3 per cent) of non-specific reactions is inevitable. A clinical analysis of such reactions covering a period of six years disclosed the fact that about 60 per cent occurred in pathologic conditions and the remaining 40 per cent in apparently normal conditions. In Table II we give the details of the percentage distribution of the nonspecific fixations.

As regards the class of cardiacs assuming that heart tissue destruction was the cause of nonspecific reactions we investigated the percentage occurrence of such reactions in forty seven cases of sundry cardiopathies clinically classified as myocardiacs and endocardiacs. The findings are given in Table III.

As the percentage of fixations was equally distributed between the two major cardiac conditions it is assumed that the rate of tissue destruction in terms of antibody production is about equal in all cardiopathies. In view of the fact that heart tissue can stimulate the production of the Wassermann reagent the above observations point strongly to the heart as the source of the antigenic substances in syphilis; nevertheless, before we can consider seriously such a possibility, we must have a better understanding of the mode of action of other than heart tissue extractives.

It is generally accepted that heart muscle extracts are the best antigens for the Wassermann reaction although other organ extractives have been more or less successfully used for the same purpose. It is quite possible that there may exist a certain degree of organ specificity depending on the origin of the lipid used as antigen, but as in other immunity phenomena the specificity is more or less generic and not strictly limited to a given organ. On the other hand, we may not exclude the possibility of a simultaneous stimulation, constructive or destructive of more than one organ by the syphilitic virus.

As we have stated the nonspecific reactions obtained in cardiacs were usually of a weak nature, not over two plus, while the specific titers in syphilis usually run very high. If the difference were only quantitative there would be little objection to accepting their natural similarity. As the production of antibodies depends on the effectiveness, the amount and the mode of administration of the antigen as well as the responsiveness of the recipient it would be expecting too much to presume two clinically dissimilar diseases syphilis and cardiopathy to give rise to a rather specific antibody in exactly the same proportion. With all this in view we still lack direct evidence of their qualitative similarity excepting the fact that both antibodies react similarly with the same antigen.

SUMMARY AND CONCLUSIONS

1. Antigens cannot be considered as homogeneous material.
2. They are composed of two serologically dissimilar molecules (a) the immunogenic, i.e., immunity exciting or antibody producing fraction, a prop

erty residing in the protein molecule, and (b) the *immunophilic*, i e, the antibody-fixing property residing in the lipin molecule

3 This theory applied to the heart muscle tissue proved correct by artificially inducing the Wassermann reagen in experimental animals by the parenteral introduction of the immunogenic fraction and the subsequent fixation of the latter by the immunophilic lipin fraction

4 The serologic changes in the course of syphilitic infection can be induced by widely dissimilar agents affecting similar organs or groups of organs and essentially the myocardium

REFERENCES

- ¹Forssmann, J Chemical Study of the Wassermann Substance and Antibodies, *Acta Path et Microb Scand*, 1924, i, 1 103
- ²Doerr, R, and Hallauer, C Antigenic Function of Lipoids, *Schweitz med Wehnschr*, 1923, lv, 962
- ³Sachs, H, Klopstock, A, and Weil Pathogenesis of Syphilitic Blood Change, *Deutsch med Wehnschr*, 1925, li, 589, The Reactivity of the Organism towards Lipoids, *Deutsch med Wehnschr*, 1925, li, 1015
- ⁴Klopstock, F Syphilitic Blood Changes, *Deutsch med Wehnschr*, 1926, lvi, 226, Syphilitic Blood Changes, *Deutsch med Wehnschr*, 1926, lvi, 1460
- ⁵Brandt, R, Guth, and Muller The Wassermann Reaction Induced by Injection of Lipoids and Serum, *Klin Wehnschr*, Berlin, 1926, v, 68, Lipoid Antibodies, *Klin Wehnschr*, Berlin, 1926, v, 655
- ⁶Much, H Biologic Significance of Lipoids, *Munchen med Wehnschr*, 1925, lxxii, 2089
- ⁷Laundau and Held Positive Wassermann Test in Slow Malignant Endocarditis, *Bull de la Soc med des Hop*, Paris, 1925, xlv, 1322

THE DUALITY OF THE ANTIGENIC NATURE OF ERYTHROCYTES

By L. G. HADJOPOULOS, M.D. and REGINALD BURBANK, M.D., NEW YORK CITY

IN a previous communication¹ we demonstrated the existence of two distinct antigenic fractions in the heart muscle tissue. First the *immunogenic* (immunity exciting) or antibody forming fraction represented in the protein molecule, and second, the *immunophilic* (the immune element) or antibody fixing fraction represented in the lipin molecule derived from the heart muscle tissue by alcoholic extraction.

The same technic was followed in the present work that was applied in fractioning the heart muscle antigen. In short it consisted in washing the erythrocytes with saline until they were absolutely free of serum proteins. The dried and pulverized material was extracted with absolute alcohol. After decanting the alcoholic, immunophilic fraction the sediment was repeatedly washed in a mixture of equal volumes of alcohol and ether until the sediment was absolutely lipin free. This end product dried and pulverized, constituted the immunogenic fraction of the erythrocytic antigen.

Rabbits were injected separately with a saline suspension of the above protein and lipin fractions. They were given in all five progressively increasing doses within a period of fifteen days. On the tenth day after the last dose they were bled. The results of their serum reactions are given in Table I.

TABLE I
IMMUNITY REACTION TITERS AGAINST FRACTIONAL ERYTHROCYTIC ANTIGEN INJECTIONS

DESCRIPTION	AGGLUTININ TITERS	HEMOLYSIS TITERS	COMPLEMENT-FIXATIONS WITH	
			LIPIN FRACTION	DELIPINIZED PROTEIN FRACTION
Rabbit A control animal	1 1	1 50	-	-
Rabbit 1 injected prot. fraction	1 10	1 5 000	++++	-
" 2 " "	1 5	1 3 000	++++	-
" 3 lipin	1 -	1 2	-	-
" 4 " "	1 1	1 50	-	-

Independent of whether the injected material was heart muscle tissue or erythrocyte the nature of findings was practically identical. The simultaneous stimulation of other immune elements as agglutinins and hemolysins was similarly expected. In a previous article² we demonstrated the production of hemolysins by injecting dehemoglobinized erythrocytes (cell stromata) and that of agglutinins by injecting cell free hemoglobin. The only departure in the present work was the use of delipinized cells (stroma plus hemoglobin) to prove that the lipin element was not necessary for the production of immune bodies.

As in our findings in the case of heart muscle tissue the antibody formed in response to delipinized cell protein injection was strongly hypotropic and com-

From the Department of Laboratories of Beth Israel Hospital, New York.
Received for publication March 3, 1927.

pletely nonproteotropic At this stage of the work the question came up, considering its mode of production, as to whether or not this antibody could display any specificity The direct experimental evidence, as given in Table II, is in favor of such specificity

The above experiments clearly indicate the specific nature of cell lipins in contrast to heart tissue lipins, i.e., the Wassermann antigen Moreover this specificity is not absolute as we demonstrate in Table III, where Wassermann positive serums were tested with both antigens

Comments—In the light of our previous findings in reference to the specificity of lipin antigens of different origins, the above tests are rather confusing unless we consider certain facts First, the titration of our cell lipin antigen

TABLE II

RESULTS OF COMPLEMENT FIXATION TESTS OF IMMUNE BODIES PRODUCED BY ERYTHROCYTIC PROTEIN INJECTIONS WITH THEIR HOMOLOGOUS CELL LIPIN ANTIGEN AND THE HETEROLOGOUS WASSERMANN LIPIN ANTIGEN

DESCRIPTION	AMOUNT SERUM C C	COMPLEMENT FIXATIONS WITH CELL LIPIN ANTIGEN	WASSERMANN ANTIGEN
Complement control (Pooled guinea pig serum)	0 04	-	-
Serum of rabbit injected with delipinized erythrocytic protein	0 0001 0 0002 0 0004	++ +++ ++++	- - -

The cell lipin antigen was titrated previously against one unit of complement. The lowest dilution that did not interfere with hemolysis was taken as the unit (in this particular case 0 50 c c of 1 40 dilution) Two units of complement were used in the tests

TABLE III

THE NATURAL ANTISHEEP HEMOLYSIS TITERS OF HUMAN SERA AND THEIR EFFECT ON COMPLEMENT FIXATION REACTIONS WITH CELL LIPIN ANTIGEN AND WASSERMANN ANTIGEN

SERIAL NUMBER	DESCRIPTION	NATURAL HEMOLYSIS TITERS	COMPLEMENT FIXATION TESTS WITH CELL LIPIN ANTIGEN	WASSERMANN ANTIGEN
	Complement control (Pooled guinea pig serum)	None	-	-
1316	Wassermann negative controls	0 02 c c	++	-
1317	" " "	0 02 c c	++	-
1318	" " "	0 02 c c	+	-
1319	" " "	0 025 c c	-	-
1320	" " "	0 02 c c	+	-
1321	" " "	0 02 c c	++	-
D M	" " "	0 025 c c	-	-
D S	" " "	0 03 c c	-	-
N K	Luetic under treatment	0 03 c c	+++	++++
J P	" " "	0 02 c c	++++	++++
1619	" " "	0 02 c c	++++	++++

was performed against guinea pig serum which is invariably devoid of anti sheep hemolysin Second, human serum invariably contains anti sheep hemolysin in fair amounts It was therefore natural to expect a certain degree of fixation of complement in the presence of a homologous antigen, the cell lipin

By comparing the natural hemolysin titer of the above serums against their homologous fixation, we found that the titer of our cell lipin antigen, 0 50 c c of a 1 40 dilution, was just sufficient to detect such concentrations of natural hemolysin as would occur in 0 02 c c of serum or less Lower con

centrations were not detected with this standard dose (In Table III compare cases 1316, 1317, 1318, 1320, and 1321, against 1319, D M, and D S) In the luetic series, with the cell lipin antigen case N K should be negative and cases J P and 1619 not over two plus As they give stronger fixations than would ordinarily be attributed to the amount of natural hemolysin present, however the question arises as to whether we should consider this as the nonspecific influence of a strongly concentrated luetic reagen on a weak sister antibody

As such phenomena are common in serologically allied substances we resorted to the absorption method of separating and handling them individually In default of a method to eliminate the luetic reagen we absorbed the natural hemolysin with sheep cells and tested the cell lipin antigen against the luetic serums minus the hemolysin The results are given in Table IV

Comments—With the complete absorption of the natural hemolysin (the antibody to sheep cell lipin) the complement fixation with the latter antigen was reduced to negative while the Wassermann reaction was unaffected As a matter of fact absorption slightly increased the Wassermann reaction

TABLE IV

THE EFFECT OF THE ELIMINATION OF NATURAL ANTISHEEP HEMOLYSIN ON THE CELL LIPIN AND THE WASSERMANN REACTION

SERIAL NUMBER	DESCRIPTION	NATURAL HEMOLYSIN TITERS	COMPLEMENT FIXATION TESTS WITH	
			CELL LIPIN ANTIGEN	WASSERMANN ANTIGEN
J P	Luetic, unabsorbed serum	0.02 cc	++++	++++
	absorbed with cells	None	-	++++
1606	Luetic, unabsorbed serum	0.02 cc	++	++
	absorbed with cells	None	-	+++
1692	Chronic, unabsorbed serum	0.01 cc	++	+
	Cardiac absorbed with cells	None	-	+

It is generally accepted that the presence of a high hemolysin titer in a luetic serum may result in a relative reduction of the intensity of the Wassermann reaction Our findings, which are not limited to the few cases above tabulated are in conflict with this view We have no satisfactory explanation for this except the possibility that during the absorption of the natural hemolysin, the addition of sheep cells introduces a nonspecific element that reacts the same way as the luetic reagen

In other respects the findings were as would be expected, namely the degree of cell lipin fixation was in proportion to the natural hemolytic titers (Cases 1606, 1692) and the presence of synergism between the luetic reagen on the cell lipin antigen as in case J P Case 1692 was expressly introduced in this table as a group representative of some minor nonspecific fixations occurring in a fair percentage of chronic cardinals, with the purpose of investigating whether such minor nonspecific Wassermann reactions were due to a reversal of the synergism between the sister antigens Basing our stand on this experiment and others that are not quoted here, we may safely conclude that the weak fixations occurring in cardinals are not at all influenced by the presence of natural hemolysins but that they are specific indicating in

a way heart tissue degeneration. This aspect of the question has already been dealt with in a previous communication.¹

SUMMARY AND CONCLUSIONS

In a previous communication we demonstrated the existence of two distinct antigenic fractions in the heart muscle tissue, the *Immunogenic* (immunity exciting) or antibody-forming fraction, a property limited to the protein molecule, and the *Immunophilic* (immune element) or antibody fixing fraction, a property of the lipid molecule derived from the same tissue. The same principle was applied in the case of sheep's erythrocytes and found strictly correct, thus demonstrating the dual nature of similar antigens.

The hemolysin, an antibody, whether produced artificially in animals or naturally present in human serums, immunologically is identical with the lipotropic antibody produced against the immunogenic fraction of red cells.

This lipotropic antibody is strictly specific, like its sister antibody produced by the parenteral introduction of the immunogenic fraction of heart muscle tissue. The coexistence of both sister antibodies, the natural hemolysin and the Wassermann reagent, displays certain synergistic relationship. The synergism, however, is not reversible. In the presence of both antibodies the natural hemolysin alone is stimulated by the Wassermann reagent, the reverse never taking place. Therefore, the weak nonspecific Wassermann reactions that occur in some cases of cardiac and cardionephritics indicate the existence of a specific lipotropic antibody produced in response to the stimulus of the immunogenic fraction of heart muscle tissue.

REFERENCES

- ¹Hadjopoulos, L. G., and Burbank, Reginald. The Origin and Nature of the Wassermann Antigen, THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, July, 1927, p. 973.
- ²Hadjopoulos, L. G. The Nature of Specific Hemolysins, Arch. Int. Med., 1921, xxvii, 241.

STUDIES IN LOCAL ANESTHESIA VI

FURTHER OBSERVATIONS OF PARA AMINO BENZOATE COMPOUNDS ON THE RABBIT EYE*

By SEYMOUR J COHEN M.S., M.D. CHICAGO, ILL.

IN A previous paper,[†] we reported the action of a series of para amino benzoate compounds in producing local anesthesia on the eyes of rabbits. These preparations were prepared by Dr. Roger Adams of the University of Illinois. He has prepared and furnished us with another series of drugs, which are also based on the structural formula of procaine and for which we have also determined the anesthetic efficiency in producing anesthesia on the rabbit eye.

In the tables are the series of drugs submitted to us for examination.

METHOD

The drugs were prepared in mol/20 concentration which corresponds to about 17 per cent cocaine solution. The method was the same as used in the previous work,—chiefly the instillation of the anesthetic solution into the conjunctival sac of a rabbit for one minute then determining the duration of anesthesia as shown by the action of the winking reflex on touching the corner with an applicator.

These results indicate that the greatest anesthetic efficiency for this series of drugs occurs when the terminal amino group contains either the ethyl, propyl, or butyl radicals. The introduction of the methyl radical in this terminal amino group causes a loss of the anesthetic action of this drug (No. 14) while the introduction of the amyl radical produces a drug that is very irritant and sometimes corrosive when compared with the same concentration of a cocaine solution (Nos. 9, 10, 17, 20). The substitution of the piperidine ring in place of the terminal amino group renders the drug much less anesthetic (Nos. 7, 8, 15, 16). The substitution of the cyclohexane radical for the alcohol portion of the molecule produces a drug with strong anesthetic properties (Nos. 22, 23, 24, 26, 28). The one phosphate salt is much less anesthetic than the hydrochloride salt. (1A) Judging from our one example the cis trans isomerism (Nos. 22 and 23) has no influence on the anesthetic value of the drug. Both are good anesthetics.

It appears that the drugs with the greatest anesthetic value and the least corrosive action are those with the normal propyl and butyl radical (No. 18) in the terminal amino group. The iso and secondary salts of these

*From Department of Pharmacology and Therapeutics, University of Illinois College of Medicine, Chicago.

Received for publication February 28, 19

JOUR. LAB. AND CLIN. MED. 19 5 xi 173

TABLE I

	NAME	FORMULA		MOL WT
1 A	β Diethylamino isopropyl para amino benzoate phosphate	$(p)NH_2C_6H_4CO_2CH(CH_3)CH_2N(C_2H_5)_2H_3PO_4$		
1	β Diethylamino isopropyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH(CH_3)CH_2N(C_2H_5)_2HCl$		348
2	β Diethylamino n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_2H_5)_2HCl$		286
3	β Diethylamino n heptyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2CH_2CH_2CH_2N(C_2H_5)_2HCl$		286
4	ϵ Diethylamino n amyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2N(C_2H_5)_2HCl$		342
5	δ Diethylamino n butyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2CH_2N(C_2H_5)_2HCl$		314
6	β Di n butyl amino n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_2H_5)_2HCl$		300
7	γ (3 carbomethoxy piperidyl)n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N\begin{matrix} H & CH_2-CH \\ & / \quad \backslash \\ & CH_2-CH \\ & \quad \\ & Cl \quad CO_2CH_3 \end{matrix}HCl$		342
8	γ Piperidyl n propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N\begin{matrix} H & CH_2-CH_2 \\ & / \quad \backslash \\ & CH_2-CH_2 \\ & \quad \\ & Cl \quad CO_2CH_3 \end{matrix}HCl$		356
9	γ Di 180 amylamino propyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_2H_{11}(180))_2HCl$		298
				370

TABLE I—CONT'D

NAME	FORMULA	MOL WT
19 β D, see butylamino ethyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(C_4H_9)(see)HCl$ $C_{17}H_{29}O_2N_2HCl$	328
20 β D, n amylamino ethyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(C_5H_{11})(n)HCl$ $C_{18}H_{31}O_2N_2HCl$	356
21 β Allyl n butylamino ethyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N\left(\begin{array}{c} H \\ \\ CH-CH=CH \end{array}\right)\left(\begin{array}{c} Cl \\ \\ CHCHCHCH_3 \end{array}\right)$ $C_{16}H_{23}O_2N_2HCl$	312
22 4 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(CH_3)_2HCl^*$ $C_{15}H_{21}O_2N_2HCl$	298
23 4 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(CH_3)_2HCl^*$ $C_{15}H_{21}O_2N_2HCl$	298
24 3 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(CH_3)_2HCl$ $C_{15}H_{21}O_2N_2HCl$	298
26 3 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(CH_3)_2HCl$ $C_{15}H_{21}O_2N_2HCl$	298
28 2 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(CH_3)_2HCl$ $C_{15}H_{21}O_2N_2HCl$	326
28 2 Dimethylamino cyclohexyl para amino benzoate hydrochloride	$(p)NH_2C_6H_4COCH_2CH_2N(CH_3)_2HCl$ $C_{15}H_{21}O_2N_2HCl$	326

*Stereoisomeric forms No -- has a melting point of 220-222 C while No -3 melts at from 263°-264° C

TABLE II

DRUG	DURATION OF ANES- THESIA IN MIN	MOL WT	REMARKS
1 β Diethylamino isopropyl para amino benzoate hydrochloride	28	286	Poor
1 A. β Diethylamino isopropyl para amino benzoate phosphate	8	348	
5 δ Diethylamino n butyl para amino benzoate hydrochloride	30	300	
4 ϵ Diethylamino n amyl para amino benzoate hydrochloride	36	314	Irritant
3 β Diethylamino n heptyl para amino benzoate hydrochloride	31	342	
14 γ Dimethylamino propyl para amino benzoate hydrochloride	0	258	Poor
2 β Diethylamino n propyl para amino benzoate hydrochloride	26	286	
11 γ Di allyl amino propyl para amino benzoate hydrochloride	28	310	
21 β Allyl n butylamino ethyl para amino benzoate hydrochloride	37	312	
12 γ Allyl n butylamino propyl para amino benzoate hydrochloride	28	326	
18 β Di n butylamino ethyl para amino benzoate hydrochloride	29	328	Irritant
19 β Di sec butylamino ethyl para amino benzoate hydrochloride	33	328	Irritant
6 β Di n butyl amino n propyl para amino benzoate hydrochloride	43	342	Irritant
13 γ Di sec butylamino propyl para amino benzoate hydrochloride	49	342	
17 β Di iso amylamino ethyl para amino benzoate hydrochloride	75	356	Corrosive
20 β Di n amylamino ethyl para amino benzoate hydrochloride	75	356	Corrosive
9 γ Di iso amylamino propyl para amino benzoate hydrochloride	75	370	Corrosive
10 γ Di n amylamino propyl para amino benzoate hydrochloride	75	370	Corrosive
15 β Piperidyl ethyl para amino benzoate hydrochloride			
16 β (3 carbomethoxy piperidyl) ethyl para amino benzoate hydrochloride	10	284	Poor
8 γ Piperidyl n propyl para amino benzoate hydrochloride	0	342	Poor
7 γ (3 carbomethoxy piperidyl) n propyl para amino benzoate hydrochloride	28	298	
	0	356	Poor
23 4 Dimethylamino cyclohexyl para amino benzoate hydrochloride	43	298	
24 3 Dimethylamino cyclohexyl para amino benzoate hydrochloride	44	298	
24 3 Dimethylamino cyclohexyl para amino benzoate hydrochloride	22	298	
26 3 Dimethylamino cyclohexyl para amino benzoate hydrochloride	42	326	Irritant
28 2 Dimethylamino cyclohexyl para amino benzoate hydrochloride	21	326	

radicals are also good anesthetics but have a tendency to cause irritation and edema of the conjunctiva (Nos 6, 13, 19)

I wish to thank Dr Jacob Sacks and Mr M Cahan for their help in this work

A NOTE UPON THE BACTERIOLOGY OF EXCISED TONSILS*

BY ROBERT A KILDUFFE,† A M, M D, ASSISTED BY W W HERSOHN,
ATLANTIC CITY, N J

WHILE it is not the presence of bacteria but the sequelae consequent upon their successful invasion of the body tissues which produce the phenomena of disease, and while the tonsils are well recognized as the habitat of a varied bacterial flora, in view of their implied and frequently proved importance as foci of infection this study of the bacteriology of excised tonsils was deemed of interest

The tonsils were received in the laboratory wrapped in sterile gauze. After searing the outer surface, streak cultures were made upon blood agar plates from which, when necessary, subcultures were made to various media for bacterial identification.

In all a total of 409 tonsils were thus examined with the results tabulated below

TABLE I
PURE CULTURES

ORGANISM	NUMBER OF TIMES FOUND
Pneumococcus (mucosus capsulatus)	3
Pneumococcus (other types)	21
M catarrhalis	9
B Friedlander	4
Staphylococcus albus	11
Staphylococcus aureus	32
Streptococcus hemolyticus	2
Streptococcus nonhemolyticus	5
	87

As all the tonsils examined were definitely diseased and the site of chronic lesions, the incidence of the various organisms is of some interest. The rather low incidence of streptococci (6.4 per cent) was somewhat surprising, the organisms most frequently found (Staphylococcus aureus, 33 per cent, M catarrhalis, 34 per cent, and pneumococcus, 54 per cent) being organisms not usually associated with those diseases often attributed to focal tonsillar infections.

In view of the fact that the tonsils in general were of chronic type, the

*From the Laboratories of the Atlantic City Hospital

Received for publication February 25 1927

†Director Laboratories Atlantic City Hospital

infrequency of pure cultures is not surprising, secondary invasions being common

The varied bacterial flora encountered emphasizes the difficulty associated with the interpretation of tonsillar cultures in the study of focal infections and also, perhaps, suggests the advisability of the routine use of the blood agar plate in conjunction with the Loeffler tube in the study of throat infections

TABLE II
TWO ORGANISMS IN CULTURE

ORGANISMS	NUMBER OF TIMES FOUND	
<i>Streptococcus nonhemolyticus</i> and <i>pneumococcus</i>	16	
<i>Streptococcus nonhemolyticus</i> and <i>Staphylococcus albus</i>	13	
<i>Streptococcus nonhemolyticus</i> and <i>Staphylococcus aureus</i>	13	
<i>Streptococcus nonhemolyticus</i> and <i>M. catarrhalis</i>	5	
<i>Streptococcus nonhemolyticus</i> and <i>leptothrix</i>	1	
<i>Streptococcus nonhemolyticus</i> and Gram negative bacillus, unidentified	3	
<i>Streptococcus nonhemolyticus</i> and <i>B. Friedlander</i>		54
<i>Streptococcus hemolyticus</i> and <i>Staphylococcus aureus</i>	2	
<i>Streptococcus hemolyticus</i> and <i>pneumococcus</i>	4	
<i>Streptococcus hemolyticus</i> and <i>M. catarrhalis</i>	1	
<i>Streptococcus hemolyticus</i> and <i>leptothrix</i>	1	
<i>Streptococcus hemolyticus</i> and Gram negative bacillus unidentified	1	9
<i>Streptococcus viridans</i> and <i>Staphylococcus albus</i>	2	
<i>Streptococcus viridans</i> and <i>pneumococcus</i>	2	
<i>Streptococcus viridans</i> and <i>M. catarrhalis</i>	2	6
<i>Pneumococcus</i> and <i>Staphylococcus albus</i>	20	
<i>Pneumococcus</i> and <i>Staphylococcus aureus</i>	24	
<i>Pneumococcus</i> and <i>M. catarrhalis</i>	36	
<i>Pneumococcus</i> and diphtheroids	1	
<i>Pneumococcus</i> and <i>B. influenzae</i>	4	
<i>Pneumococcus</i> and <i>leptothrix</i>	1	
<i>Pneumococcus</i> and Gram negative bacillus unidentified	19	
<i>Pneumococcus</i> and Gram positive bacillus unidentified	1	106
<i>Staphylococcus aureus</i> and <i>Staphylococcus albus</i>	14	
<i>Staphylococcus aureus</i> and <i>M. catarrhalis</i>	1	
<i>Staphylococcus aureus</i> and Gram negative bacillus, probably <i>B. Friedlander</i>	6	
<i>Staphylococcus aureus</i> and Gram positive bacillus, unidentified	1	
<i>Staphylococcus aureus</i> and <i>B. proteus vulgaris</i>	1	23
<i>Staphylococcus albus</i> and <i>B. Friedlander</i>	5	
<i>Staphylococcus albus</i> and Gram negative bacillus, unidentified	3	
<i>Staphylococcus albus</i> and <i>B. influenzae</i>	1	
<i>Staphylococcus albus</i> and <i>M. catarrhalis</i>	8	
<i>Staphylococcus albus</i> and diphtheroids	1	18
<i>M. catarrhalis</i> and diphtheroids	1	
<i>M. catarrhalis</i> and <i>B. Friedlander</i>	6	
<i>M. tetragenus</i> and <i>B. Friedlander</i>	3	10

TABLE III
THREE ORGANISMS IN CULTURE

ORGANISMS	NUMBER OF TIMES FOUND	
Streptococcus viridans, Streptococcus nonhemolyticus and Staphylococcus aureus	1	
Streptococcus hemolyticus, pneumococcus, and Staphylococcus aureus	2	
Streptococcus nonhemolyticus, pneumococcus, and leptothrix	1	
Streptococcus nonhemolyticus, pneumococcus, and diphtheroids	1	
Streptococcus nonhemolyticus, pneumococcus, and M catarrhalis	7	
Streptococcus nonhemolyticus, pneumococcus, and Staphylococcus aureus	10	
Streptococcus nonhemolyticus, pneumococcus, and Staphylococcus albus	1	
Streptococcus nonhemolyticus, pneumococcus, and B Friedlander	1	
Streptococcus nonhemolyticus, M catarrhalis, and leptothrix	1	
Streptococcus nonhemolyticus, M catarrhalis, and diphtheroids	1	
Streptococcus nonhemolyticus, M catarrhalis, and Gram positive bacilli, unidentified	1	
Streptococcus nonhemolyticus, Staphylococcus aureus, and Gram positive bacilli, unidentified	2	
Streptococcus nonhemolyticus, Staphylococcus aureus, and diphtheroids	1	
Streptococcus nonhemolyticus, Staphylococcus albus, and B Friedlander	4	
Streptococcus nonhemolyticus, Staphylococcus aureus, and B Friedlander	5	
Pneumococcus, Staphylococcus aureus, and B Friedlander	5	
Pneumococcus, M catarrhalis, and B Friedlander	17	
Pneumococcus, M catarrhalis, and leptothrix	2	
Pneumococcus, M catarrhalis, and diphtheroids	6	
Pneumococcus, M catarrhalis, and Staphylococcus aureus	10	
Pneumococcus, M catarrhalis, and Staphylococcus albus	5	
M catarrhalis, Staphylococcus aureus, and Gram negative bacillus, unidentified	2	
M catarrhalis, Staphylococcus aureus, and diphtheroids	1	87

TABLE IV
FOUR ORGANISMS IN CULTURE

ORGANISMS	NUMBER OF TIMES FOUND	
Streptococcus nonhemolyticus, Staphylococcus aureus, M catarrhalis, Gram negative bacillus, unidentified (probably B Friedlander)	3	
Streptococcus nonhemolyticus, pneumococcus, M catarrhalis, Gram negative bacillus, unidentified (probably B Friedlander)	2	
Pneumococcus, diphtheroids, M catarrhalis, and Staphylococcus aureus	3	
Pneumococcus, Staphylococcus albus, M catarrhalis and leptothrix	1	9
Total cultures	409	

TABLE V
PERCENTAGE INCIDENCE OF BACTERIA FOUND

ORGANISM	NUMBER TIMES FOUND	PER CENT
Streptococcus viridans	7	1.4
Streptococcus hemolyticus	13	3.0
Streptococcus nonhemolyticus	93	20
Leptothrix	8	0.9
M catarrhalis	142	34.0
Diphtheroids	15	3.0
B influenzae	5	1.0
Pneumococcus	224	54.0
B Friedlander	61	14.0
B proteus	1	0.2
Staphylococcus aureus	136	33.0
Staphylococcus albus	7	1.7
Gram positive, unidentified bacilli	4	0.9
Total	757	

THE ACTION OF INDOL AND SKATOL ON THE HEART*

By J A WADDELL, M.D., AND J A CALHOUN A.B. CHARLOTTESVILLE, VA

INDOL and skatol are described in works on biologic chemistry and physiology as being poisonous protein derivatives which are formed in the intestine by bacterial action. To them is attributed the characteristic odor of the feces. On absorption from the alimentary canal, they are normally detoxicated, in the liver, by conjugation with certain metabolites such as sulphuric and glycuronic acid.

They are referred to in clinical literature as factors in the causation of the symptoms observed in intestinal stasis, wound suppuration, and focal infection. While the part they play in such conditions is not definitely established, the fact that they are present coupled with the evidence that they are toxic makes a strong case against them.

The most outstanding investigations of their physiologic effects are those of Rovighi¹ on indol and skatol, of Herter² on indol and of Salant and Kleitman³ on skatol. The first mentioned experimented on rabbits and other mammals and noted, among various effects, feeble heart action. The second author observed in dogs and rabbits respiratory depression, general prostration and circulatory depression. Salant and Kleitman, using cats and dogs, reported central nervous system and circulatory depression, the latter tending to persist in the case of the dog.

All of the above employed intact animals and dealt with the grosser features of the intoxication. Though each reported depression of the circulation, they noted coincidently changes in the nervous apparatus and in respiration, as well as in other systems. Which effects were primary and which were secondary was not worked out. None of them examined the heart as a separate entity, nor did they definitely establish a cardiac action on the part of these substances, although Herter does cite an experiment in which the death of the experimental animal seemed to be cardiac.

In view of the rather fragmentary state of the knowledge regarding the effects of indol and skatol on specific organs, particularly the heart, we have undertaken this investigation of their cardiac actions individually and in combination in various proportions on cold and warm blooded animals. Our study is limited to the cardiac effects, uncomplicated by changes in the blood vessels, the nervous system or the respiration.

*From the Pharmacological Laboratory of the Medical School of the University of Virginia.

Received for publication March 7, 1917

METHODS AND MATERIALS

The freshly excised hearts of frogs, turtles, cats, and rabbits were used in these experiments. The tracings were taken from the apex. A slightly different procedure was necessary for the cold blooded as compared with the warm.

In the case of the frogs and turtles, the procedure for perfusing was the same as that employed in a previous paper⁴. It consisted, in brief, in admitting the perfusate through the vena cava and securing the outflow from the aorta. A uniform temperature and constant pressure were maintained. The

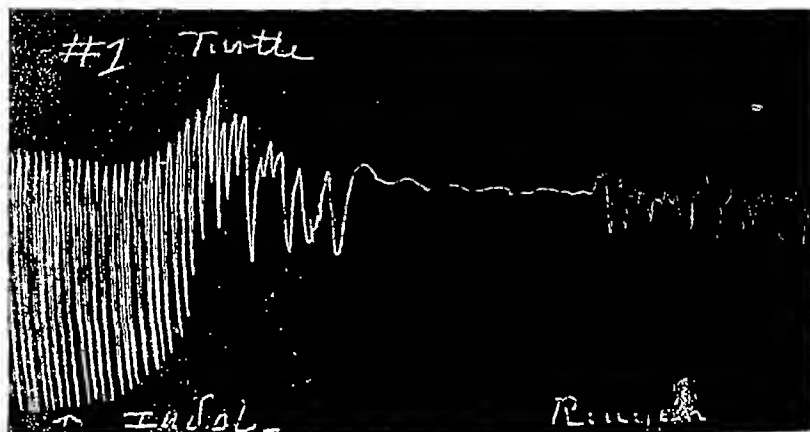


Fig 1,—Tracing 1 Heart of turtle showing the effect of half saturated Indol with partial recovery after removal

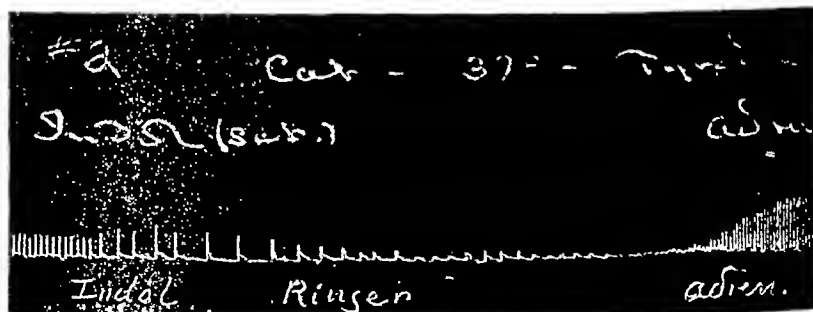


Fig 2—Tracing 2 Heart of cat showing the effect of saturated Indol with antagonism by epinephrine

study was begun in the early fall and continued into the winter. The animals were kept in the laboratory for twelve hours before the experiment, in order not to be subjected to a sudden change in temperature just prior to the observations. Ringer's (Howell) solution was employed, at room temperature 20-22° C and at 27° C.

The hearts of cats and rabbits were perfused with Tyrode's solution at 37° C. A modified Langendorff method was employed, the perfusate being admitted through the tip of the left auricle and the outflow secured from the aorta. The right auricle was punctured to permit its emptying fluid received through the coronary circulation.

As is well known, indol and skatol are very slightly soluble. Chemical tables of solubilities do not state the degree. Hence, the strengths of the solutions we used cannot be expressed in per cent, accordingly we have employed the terms, "saturated solution" "half saturated" etc, to indicate the comparative concentrations which of necessity were extremely dilute.

The following brands of the drugs were employed. Skatol from Eimer & Amend, Kahlbaum, and Eastman, Indol from Eimer & Amend, Theo. Schuchardt and Kahlbaum. One Kahlbaum and the Suchart preparation were old and quantitatively less effective otherwise all acted alike.

Our solutions were prepared twelve hours in advance by adding the drug in excess to Ringer's or Tyrode's solution and applying a temperature of



Fig 3—Tracing 3 Heart of turtle showing the effect of half saturated skatol with antagonistic action of indol



Fig 4—Tracing 4 Heart of rabbit showing the effect of saturated skatol with recovery on removal

60° C for fifteen minutes. These saturated solutions were then filtered—that in Ringer's at room temperature and that in Tyrode's at 37° C. From the filtrate the working dilutions were made.

EXPERIMENTAL DATA

The experimental data will be presented under the following captions (1) Indol (2) Skatol (3) Indol and Skatol with Other Drugs (4) Indol and Skatol with One Another and (5) Indol and Skatol on Other Nonvoluntary Muscles.

1 Indol—On the hearts of the turtle and frogs all strengths of indol down to one eighth saturated solutions were effective in slowing the heart

and in decreasing the output per minute and per beat. The latent period was relatively short. The lowest concentrations produced chiefly slowing, intermediate ones, slowing with usually a gradual decrease in diastole and occasionally grouped beats, and the highest, arrest, usually systolic, which was preceded by a few extrasystoles. The aortic stopped before the ventricle. The arrested heart responded to mechanical and electric stimuli and to epinephrine. On perfusion with plain Ringer's fluid, the effects of indol were almost entirely abolished. Automaticity was reestablished in the quiescent with peristaltic phenomena very pronounced initially. The force of the contractions was not restored. The A-V interval was not changed and heart

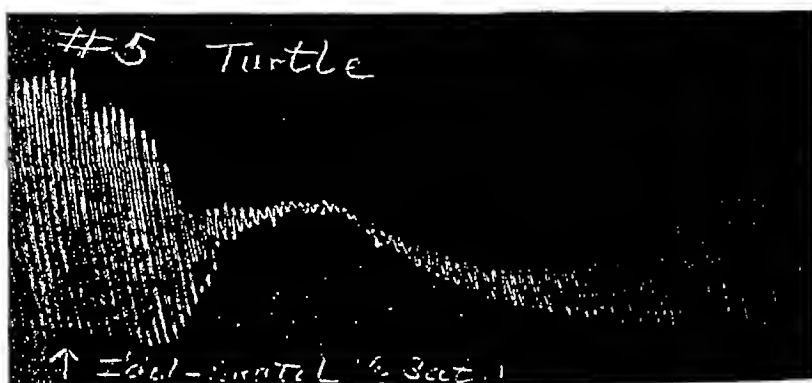


Fig 5—Tracing 5 Heart of turtle showing the effect of a balanced mixture of indol and skatol with recovery on removal

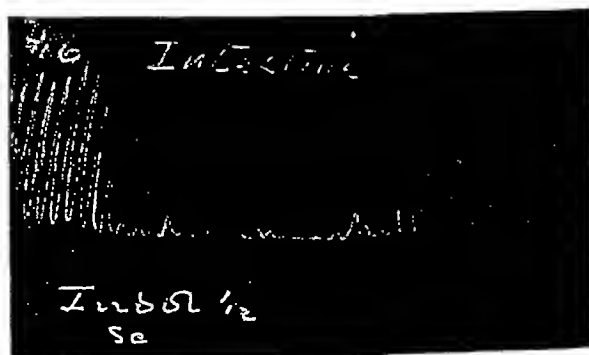


Fig 6—Tracing 6 Intestine of rabbit showing the effect of half saturated indol with partial recovery on removal

block was not exhibited. Quantitatively, the effects were more marked at 27° C than at 20° C and on reapplication than initially.

On the hearts of cats and rabbits, solutions saturated at 27° C produced almost immediate arrest of both chambers in diastole, usually, one-half saturated, only slowing with decreased amplitude. Withdrawal of the drug was rapidly followed by almost complete restoration. The decreased relaxation, observed in the turtle, was not exhibited in the case of the warm blooded heart and systolic arrest was but rarely observed.

2 *Skatol*—On the heart of the frogs and turtles, all strengths of skatol down to one-eighth saturated solutions were effective in slowing the heart

and decreasing the output. The latent period was long compared with that of indol. The lowest concentrations produced slowing, intermediate ones, slowing with usually a gradual decrease in systole, and the highest, arrest, which was usually diastolic. The auncle stopped before the ventricle and its cessation was preceded by marked dilatation. The arrested heart responded to electric and mechanical stimulation and to epinephrine. Prolonged perfusion with plain Ringer's restored the rhythm but not the force of the contractions. The drug was more effective on reapplication. The A V interval was not altered and heart block was not observed. The drug was somewhat more effective at 27°C than at 20°C as was also the case with indol.

On the hearts of cats and rabbits solutions saturated at 27°C produced tardily an arrest of both chambers in diastole, one half saturated solutions,



Fig 7—Tracing 7 Intestine of rabbit showing the effect of saturated skatol with partial recovery on removal



Fig 8—Tracing 8 Heart of frog showing the effect of Latin with recovery on removal.

only slowing with decreased amplitude, less than after indol. The decrease in the height of the concentrations observed in the turtles was also shown in the warm blooded hearts.

3 Indol and Skatol with Other Drugs—Possible interactions with epinephrine and atropine were investigated. The former was antagonistic, the result being the algebraic sum. Atropinizing did not prevent nor abolish their actions or qualitatively alter them.

4 Indol and Skatol with One Another—The effects of indol and skatol have been shown to be very similar. This was to be expected from a consideration of their close chemical relationships. The most noteworthy difference is in there usually being exhibited in cold blooded hearts after indol a

decrease in relaxation and after skatol a decrease in contraction. In view of this it would seem that they might be antagonistic in some respects, at least on the amphibian heart. Hence, they were employed in the following further experiments on the turtle (a) in sequence and (b) mixed together in various concentrations.

(a) In sequence. On changing immediately for instance from indol to skatol, the diastolic shortening of the former was altered to the systolic shortening of the latter. The effect was always that of the drug last used, though somewhat reduced. The change was exhibited sharply, no intermediate balancing of effects was observable. (See Fig 3.)

(b) Mixed together. Since indol and skatol are not nicely quantitative in their effects, the endeavor was made, by trial on individual hearts, to mix them in balanced proportions, i.e., if skatol one-fourth saturated produced approximately the same decrease in systole as indol one-fourth did in diastole, then the perfusate was prepared by mixing equal proportions of half saturated solutions. When this was effected, the combination produced a gradual decrease in both systole and diastole. With arresting concentrations, the writing point stopped in a midway position. In balanced mixtures, then, each drug exhibited its individual effect.

In the case of rabbits and cats, mixtures of indol and skatol produced simple summation. This was to be expected in view of similar actions individually.

5 *Indol and Skatol on Other Nonvoluntary Muscle*—In studying the effects of indol and skatol on other nonvoluntary muscle, the following organs were examined, suspended in Tyrode's solution at 37° C. Intestine of the rabbit and the rat, uterus of the rabbit, and the vagina of the rabbit. Indol and skatol produced identical effects, depressing the musculature of all. One eighth saturated solutions produced slowing of the rhythm without appreciable change in tone or amplitude, one-fourth saturated, decrease in both rate and amplitude, one-half saturated, depressed tone, rate, and amplitude, while saturated, a sharp drop in tone and a decrease in amplitude and rate progressing to quiescence. Removal of the drug was followed by almost, but never complete, recovery of all the properties of the tissue.

DISCUSSION

It has been shown that indol and skatol are cardiac depressants, their effects being almost identical qualitatively. Indol appeared to be more rapid and more potent in its effects, but these differences are no doubt due to its greater solubility and diffusibility, though the methyl group of skatol may have been a factor.

Both drugs act independently of the nervous apparatus and hence must be direct muscular depressants. This view is supported by the observation that they depress nonvoluntary muscle in general as is evinced in the case of the gastrointestinal tract and the reproductive organs.

We can offer no explanation of the differences shown by indol and skatol on the turtle's heart, i.e., the decreased relaxation with indol and the decreased contraction with skatol. It was the usual phenomenon during the

early fall months but was constantly exhibited in the middle of the winter. Apparently it depended on the state of nutrition of the animal, those of the autumn being freshly caught while the winter ones had been kept under artificial conditions for about two months. This will be investigated further when material becomes available.

Other species did not exhibit definite qualitative differences as regards the two drugs, nor did other tissues. It may be noted here that isatin,⁶ which has the same constitution as indol except for the replacement of two hydrogen atoms by oxygen depressed all the functions of smooth muscle and gave the same effects as indol and skatol on the warm blooded hearts, but decreased both systole and diastole in the cold blooded like a balanced mixture of indol and skatol, the two drugs with which we are dealing in this paper.

The literature on indol and skatol from the experimental standpoint was reviewed above. The several observers cited noted circulatory depression but were not explicit as to the part played by the heart. We have shown them to be direct cardiac depressants.

SUMMARY

1 Indol and skatol decrease the amplitude and rate and even arrest the hearts of frogs, turtles, rabbits, and cats.

2 Indol and skatol affect the hearts of cold blooded animals more actively than those of warm.

3 Indol is more effective than skatol and its latent period is shorter.

4 Indol and skatol do not give nice quantitative results—on one animal a one fourth saturated solution may produce as great a response as a one half saturated on another of the same species.

5 Indol and skatol become more effective on repetition, low concentrations produce (but tardily) the same effects as high and withdrawal is not followed by complete recovery.

6 Indol and skatol are antagonized by epinephrine but not by atropine.

7 Indol and skatol differ qualitatively on the turtle's heart in that the former usually decreases relaxation and arrests in systole, while the latter usually decreases contractility and arrests in diastole.

8 Indol and skatol in sequence are antagonistic, but, mixed in balanced proportions, each exhibits its peculiar effect, both systole and diastole being decreased.

CONCLUSIONS

1 Indol and skatol are cardiac poisons.

2 They are cumulative in their action.

3 They act directly on the muscle substance.

REFERENCES

- ¹Rovighi: *Semaine Medicale* 1895, xv, 466.
- ²Herter: *New York Med Jour* 1898 lxxvii 59 *Med. Rec* 1898 lxx 569.
- ³Salant and Kleitman: *Jour Pharmacol and Exp Therap* 1922, xix 293.
- ⁴Waddell and Cohen: *JOUR LAB AND CLIN MED*, 1924 ix, 3.
- ⁵van Nostrand's Chemical Annual 1919 pp 312 and 358.
- ⁶Waddell: Unpublished Data, 1926 Pharmacol Lab University of Virginia.

LABORATORY METHODS

A UNIVERSAL ARTIFICIAL RESPIRATION AND CLOSED ANESTHESIA MACHINE*

By D E JACKSON, M D, CINCINNATI, OHIO

THE device here shown can be used both for artificial respiration and for anesthesia by the closed method. The machine will give either positive or negative pressure and either of these can be either intermittently interrupted or used as a constant pressure. There are three rates of interruptions (by a lever gate valve) which give a good range for intermittently inflating the lungs of the usual laboratory animals. The volume of air discharged at each interruption by the valve can be varied from zero up to a volume sufficient perhaps for a horse. And when constant pressure is used it can be varied in the same way.

By reference to the illustration it may be seen that the machine is constructed about a heavy iron framework made up of four heavy angle iron posts (1) which are curved outward at the bottom and thus serve as a base on which the machine rests. The four posts are firmly held together by two iron plates (2 and 3) and by an iron frame (4) at the bottom. A very accurately made (and tight) Crowell rotary air pump (5) rests on the plate (3) and serves to circulate the air (or anesthetic substances) through the closed system of tubes and reservoirs. A strong motor (26) turns the pump by means of the belt (25). A reversing switch is located just behind the motor, so that if the motor is turned forward the outlet (22) will deliver positive air pressure but if the motor be reversed this outlet will register negative pressure which may be used for aspirating the chest (for lung tracings, etc) or for suction filtration, etc.

An interrupting valve (21) is located just behind the outlet tube (22). This interrupting valve is operated by a special mechanism driven by the cone pulley (23) which is fastened on the end of the pump spindle. This pulley has three grooves for the round belt (24) which drives the interrupting mechanism (not shown in the illustration).

From the positive side of the pump the air is driven through pipe (12) down into the large tubular reservoir (14) from which the compressed air escapes through tube (13) which leads to two valves, the first being the wheel valve (27) (which when opened will allow air to escape under constant pressure for blast lamps, etc) and the second the four-way valve (18). This four-way valve is operated by means of the handle (19). When the handle is turned as shown in the illustration the compressed air passes from tube (13)

*From the Department of Pharmacology, University of Cincinnati Medical School
Received for publication Feb 7 1927

upwards, then across and downwards through the four way valve and by way of tube (15) to the bottom of the canister (16). The canister contains soda lime. Or, if desired a second canister holding a saturated solution of sodium hydrate and carrying a special water trap and water gauge may be substituted in place of the canister (16) which is held on by means of the screw latches (28—28). There are some advantages in favor of using the solution but there are other advantages in favor of the dry lumps of soda lime.

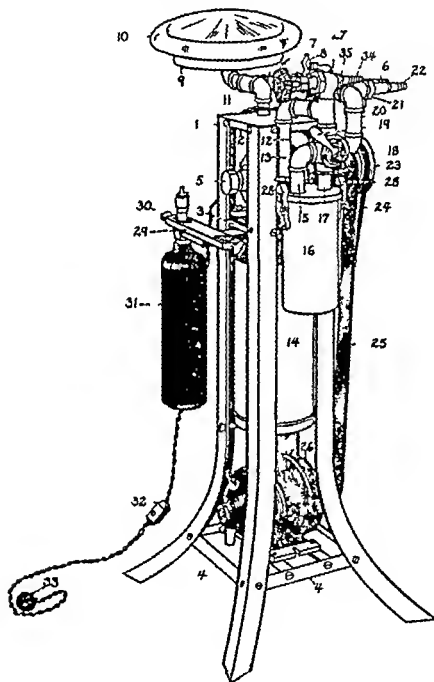


FIG. 1

From the canister (16) the air (minus the carbon dioxide if the closed system is being used) passes out through tube (17) and again through the four way valve (18) into the tube (20) which leads to the interrupting valve (21) and thence through the outlet (22) to the tracheal cannula. By turning the handle (19) of the four way valve forward the canister is cut out of the system and the air passes directly from the reservoir (14) out through the outlet (22). In this way one can either filter out the CO_2 or allow it to accumulate in the system as may frequently be desired in experimental work.

Air enters the machine through the inlet shown at (6). This inlet tube

bends around, opposite and behind the wheel valve (27), and is continued as tube (7) which leads into the tube (11). This tube (11) connects below with the negative side of the pump (5) and above, by means of a tapered slip joint, with a large shallow pan (9) over the rim of which is stretched a bath cap (10). If desired the pan can be removed (at the slip joint) and a special L-tube, over which an ordinary anesthesia bag can be fastened, may be slipped in its place and used as the flexible reservoir in place of the (cheaper) bath cap. In either case the flexible reservoir serves to accommodate the breathing of the animal when the closed system is used. If the machine is used only for artificial respiration with air, or for blast lamps, negative filtration, etc., then neither the bath cap (nor anesthesia bag) nor canister (16) need be used. If the closed system is being used and too much gas (oxygen, ethylene, etc.) is run into the machine the excess may be quickly emptied out while the pump is running by slightly opening the wheel valve (27) for a few seconds.

Just behind the wheel valve (27) a small inlet air cock (8) passes into the inlet tube (6 * * * 7). Through this air cock ethyl chloride, ether, chloroform, etc., or oxygen, acetylene, etc., from a separate cylinder may be injected at any time.

Between the course of the outlet tubes (20 * * * 21) and the course of the inlet tubes (6 * * * 7) there is a connecting tube (35) in which is placed a by-pass valve. The handle of this valve (not numbered) is shown just above the wheel valve (27). When the by-pass valve is closed all the positive air pressure passes out through the outlet (22). But if the by-pass valve be wide open all the positive air pressure will pass through the connecting tube directly over into the (suction) inlet tube (6 * * * 7) and thence back into the pump. But if the by-pass valve is half open then half the positive pressure will pass out at the outlet (22) and half will pass back into the inlet side (negative pressure) of the system. By varying the amount of air passing through the by-pass any amount (volume) of air desired may be blown out at the outlet (22). Thus exactly the necessary amount of lung inflation can be secured. The by-pass valve acts in the same way if the motor be reversed and suction through the tube (22) be employed (for aspirating the chest in making lung tracings, etc.).

A set screw (30) serves to hold a cylinder (31) of oxygen, ethylene, nitrous oxide, propylene, etc., in the double yoke bar (29) which is supported from the plate (3). A small drilled hole and tubing carry the gases from the cylinders into the large reservoir (14).

Electric current for the motor (110 volts alternating or 110 volts direct—the proper $\frac{1}{4}$ horse power motor must be supplied for each current) is obtained through an extension cord shown at the left in the figure. The plug (33) attaches to a lamp socket or other outlet and the current is controlled by the switch (32).

The tubing used in the machine is brass and all joints are soldered. The supporting plate to which the canister (16) is attached carries a rubber gasket on its lower surface and the upper rim of the canister is drawn up tightly against the gasket and plate by means of the set screws in the latches (28—28). Thus the closed system can be made an tight throughout.

If the machine is used only for inflating the lungs with air as in ordinary artificial respiration, then a rubber tube $\frac{1}{4}$ inch in diameter and two or three feet long is attached to the outlet (22) and carried over to the animal where it is attached to the tracheal cannula. The tracheal cannula is best made in the form of a T tube and the rubber tube leading from the respiration machine is attached to the side tube of the cannula. The straight end of the cannula (away from the animal) carries a short piece of rubber tubing on which is placed an adjustable screw clamp. By means of this clamp any excess of air which the machine would blow into the lungs may be allowed to escape and the degree of lung inflation can be controlled exactly as desired.

If, however, it is desired to use the closed system (with ethylene, etc.) a slight modification of technique is necessary. In this case two rubber tubes lead from the machine (outlet 22 and inlet 6) to the animal. If the animal is not to be operated upon and the trachea is not to be opened then a metal mask carrying a heavy perforated rubber membrane (through which the animal's nose is thrust) is strapped tightly to the animal's head. A tubulature in the mask carries a large, perforated cork. Through the hole in this cork is thrust one end of a special tracheal cannula. This cannula is of the T tube variety but has two side tubes instead of one. The rubber tubes from the respiration machine are attached to the two side tubes of the cannula. Then one end of the cannula is passed through the cork in the mask and a short piece of rubber tubing carrying a screw clamp is used to close the distal end of the cannula. The mask is then placed on the animal and the machine started. The interrupting valve (21) need not be used in this case and is thrown out of action. The machine now merely circulates the anesthetic mixture round and round the CO_2 being filtered out as desired.

But if one desires he may quite easily open the chest and still use the closed system, employing ethylene, etc. as the anesthetic. This is done by a slight modification of the above technique. The double side outlet tracheal cannula is used. But in this case a small screw clamp is placed on the end of the negative rubber tube near to the side tube of the tracheal cannula and the distal end of the cannula is closed tightly. If the cannula is now tied tightly into the trachea (instead of being pushed through the hole in a cork as in the above technique) it will be seen that air will be blown from the machine through the positive tube to the tracheal cannula and thence into the lungs. But if the (negative) tube leading from the tracheal cannula back to the inlet of the machine is of the same size as the tube which carries the current of air from the machine into the cannula, then most of the air will simply circulate around through the cannula and go directly back through the inlet tube into the machine without having inflated the lungs at all. This difficulty is overcome by simply partly closing the screw clamp on the negative rubber tube very close up to the side tube of the tracheal cannula. This makes it more difficult for the air to pass out of the cannula back to the machine and since an excess of air is blown by the machine into the cannula at each intermittent discharge it is seen that the lungs will be inflated by this excess which just as soon as the machine ceases to discharge air from the positive tube,

will again pass back (a little more slowly) through the constricted part of the tube (where the screw clamp is partly closed) and on into the machine. By properly adjusting the screw clamp the degree of lung inflation can be controlled perfectly, either with the chest opened or closed.

If desired an ordinary etherizing bottle may be attached to the inlet tube (6) and the ether vapor thus drawn into the machine can be used to maintain the anesthesia.

The machine has been designed to cover a very wide range of experimental work, and if desired a recording spirometer can be attached (at the slip joint) in place of the bath cap or anesthesia bag. The machine has a capacity which is more than ample for experimental work on man, and I have no doubt but that it could be used for this purpose although I have not as yet carried out any such experiments.

For many years I have used in one device or another all of the principles involved in this machine. At the October meeting, 1923, of the anesthetists in Chicago I described a machine (with lantern slides) which did exactly the work which this machine does. And the earlier machine I also demonstrated in experiments carried out in June, 1924, in Prof. McGuigan's laboratory in Chicago. Recently¹ Prof. Starling in England has described an artificial respiration machine which involves some of the principles used in this machine.

The machine as here described may be obtained from the Max Woehner & Son Co., Cincinnati, Ohio.

REFERENCE

- ¹Starling, E. H. Jour. Physiol., March 18, 1926, LV (Proceedings of the Physiological Society for Jan. 23, 1926, p. LV.)

GASTRIC MOVEMENTS IN THE PIGEON WITH ECONOMY OF ANIMAL MATERIAL COMPARATIVE STUDIES V*

By T. L. PATTERSON A. M. M. S., Ph. D., DETROIT, MICH

A FEW years ago I¹ published a method for studying the movements of the empty and filled stomach of the bullfrog which had given a certain degree of satisfaction in the hands of laboratory students. As pointed out in this article the methods as applied to the study of the movements of the gastrointestinal tract both in hunger and digestion have been carried out largely on man and the higher laboratory animals. In larger animals such as dogs with gastric fistulae a study of peristalsis can only be made after a period of intensive training and under the quietest surroundings.

Rogers² has shown that the contractions of the empty crop of the pigeon may be studied either by the balloon method or by direct observation. In the normal bird these contractions are not easy to demonstrate except by the balloon method, since the hungry animal in a cage is in a state of restless excitement, in which it can be seen only that the crop is empty. If such a bird be quieted, however, in a partially darkened cage with the observer sitting quietly at the side the contractions of the empty crop can be seen without any form of registering apparatus. This demonstrates that the presence of the balloon in the crop does not necessarily act as the stimulus to the contractions and overcomes the objection of some to the balloon method for this type of work. The failure of Rossi³ and Dojon⁴ to control the inhibitory influences is probably one reason for these investigators claiming that the empty crop is quiescent. The placing of blinds over the bird's eyes usually has the same quieting influence as darkening the cage. Under these conditions with the bird quiet, one is able to observe at intervals one or more deep peristaltic waves running over the entire crop and this is frequently the precursor of the bird becoming restless. At other times, instead of the periodic contractions the entire crop may be so constricted as to nearly obliterate its lumen. This indicates that there is a relation between tonus and distension as described by Cannon for the mammalian stomach since peristaltic contractions will not appear on such a constricted organ. Between this high degree of constriction and that of a partially relaxed crop over which run deep peristaltic waves there may be found all intermediate gradations in the same bird at different times.

The preceding facts which with patience can be observed in the normal bird are more readily demonstrated in the decerebrate animal, for here the inhibitory influences are at a minimum. The behavior of the crop of the operated bird is practically identical with that of the normal, with the exception, that the gastric activities are no longer related to distant influences.

*From the Physiological Laboratory of the Detroit College of Medicine and Surgery.
Read before the Fortieth Annual Meeting of the Iowa Academy of Science at Cedar Rapids Iowa, April 30 and May 1 1906*

The procedure involves etherization of the bird and surgical removal of all the forebrain anterior to the thalamus. Elaborate aseptic precautions in pigeons are unnecessary. The chief difficulty is in removing all of the forebrain without causing injury to the thalamus and cerebellum or injuring the cerebral circulation. The technic employed after etherization, consists of clipping the feathers from the top of the head and exposing the cranium by a longitudinal incision through the skin. With a scalpel, a small opening is drilled through the bone on either side overlying the cerebral hemispheres of sufficient size to admit the point of a scissors blade and the bone is then carefully removed, with the exception of that portion directly over the median sinus. The dura should not be torn during this process. Then with fine pointed scissors an incision is made through the dura over both hemispheres. All this can be done practically without bleeding. A probe with the point curved to fit the posterior border of the cerebrum is then introduced under the dura and the brain substance removed, one side at a time, while the dura



Fig 1—Dorsal aspect of head of pigeon after bone excavation and removal of cerebral hemispheres. Note the size and position of the bone openings, the bridge of bone in center protecting the median sinus and the mid and hind brain showing within the posterior portion of the cavity.

is left intact. Hemorrhage is controlled by gently packing with cotton. When the bleeding subsides, the cotton is removed, leaving the cavity empty and the skin is sutured over the bridge of bone which protects the median sinus (Fig 1). A thin coat of collodium over the incision completes the procedure.

A fistula is now made in the crop directly following the decerebration and before the animal recovers from the anesthetic. The procedure consists of clipping off the feathers close to the skin from the lower end of the neck and the upper part of the breast. A small incision is made through the skin and the muscularis of the crop. This incision forms the fistula into which is inserted a piece of soft rubber tubing about two inches in length and one quarter inch in diameter, the tubing being slightly larger than the opening, thus putting the tissue around the fistula on the stretch and making secure the tube. No sutures are required. The crop end of the tube is cut obliquely while

the exterior end is closed with a cork which is made secure by a safety pin and this also prevents the tube from being drawn into the crop. If the tube is removed the fistula will close in two or three days and the animal is none the worse for the operation. Direct observation shows that such fistulae do not modify, or only vary slightly the normal movements of the crop. In twenty four hours the fistula tube may be removed and a rubber balloon introduced into the crop and connected with a water manometer for graphic registration of the movements (Fig 2). A balloon about 4 by 4 cm of the condom type is used and is tied with a silk thread onto the end of a flexible rubber tube about 4 mm outside diameter which contains a small metal or glass cylinder about 8 mm in length of such a size as to exactly fit into the lumen of the tube.

The gastric apparatus of the pigeon is anatomically divided into three parts the crop, usually considered as a simple dilatation of the esophagus

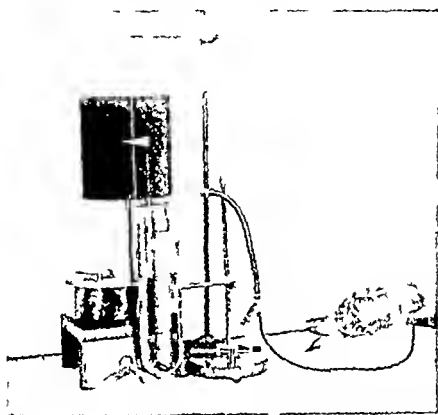


Fig —Typical decerebrate pigeon feathers raised head drawn in to shoulders eyes closed with balloon in crop connected with registering apparatus

and similar in structure to it⁶ the proventriculus or glandular stomach and the gizzard or muscular stomach. All parts of this apparatus exhibit motility and according to Kato⁷ the pressure exerted by the contractions of the gizzard during hunger are greater than those occurring after feeding. These contractions may be obtained by simply pushing the balloon into the gizzard through a fistula made in the midline of the crop. From the standpoint of comparative anatomy, the crop of the bird may also be considered to correspond to the cardia of the stomach of higher animals.

The hunger contractions obtained from the empty crop like those obtained from the gizzard in fasting are more vigorous than the digestive peristalsis after feeding. The hunger contractions of the empty crop exhibit a definite periodicity characteristic of the behavior of the empty stomach of higher animals (Fig 4). Sometimes the contractions are rapidly and con-

tinuously repeated for several hours, but they usually occur in groups periodically. On the contrary, the movements of the filled crop are of less amplitude, more irregular and less indicative of a definite periodicity (Fig 5). After feeding the hunger contractions are usually entirely absent for thirty to forty-five minutes. Then at short intervals, irregular contractions begin to occur which after an hour or two gradually increase in frequency and vigor, first appearing in groups of three or four waves and then after five or six hours in groups of six to twelve or more, separated by intervals in which the crop is comparatively at rest. It requires from ten to twenty seconds for each peristaltic wave of the empty crop to complete its cycle, whereas the more rapidly repeated contractions of the lower part of the crop occur at the rate of eight to ten per minute. If the bird sets up a shuddering side

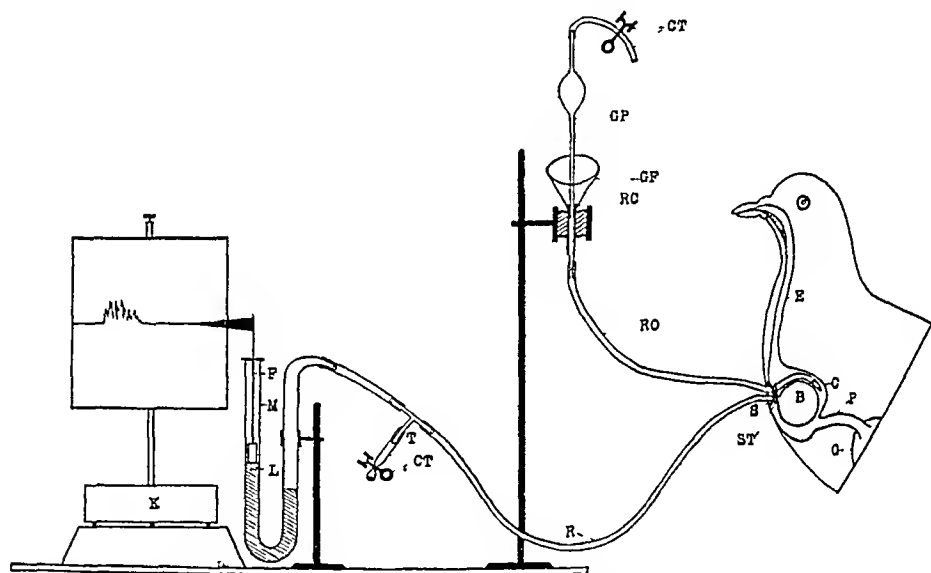


Fig 3—Diagram showing method of recording peristalsis of crop of pigeon. K kymograph, F, glass float with recording flag, M, manometer, L, manometer liquid (water), T, glass T-tube for inflation of balloon, R, rubber tube connecting balloon with manometer, S, crop fistula, B, rubber balloon in crop, E, esophagus, C, crop, P, proventriculus, G, gizzard, CT, clamp, and rubber tube, GP, glass pipette, RC, rubber cuff on end of pipette, GF, glass funnel, RO, rubber tube with open end in crop, ST, silk thread holding tubes together.

wise movement it indicates overdistension of the balloon or that the balloon is too large and it then becomes a source of irritation. Sometimes a similar condition results from overdistension of the crop with food.

For studying the influence of inhibitory substances (liquids) on the movements of the empty crop or the stomach of other animals, a second rubber tube is attached to the basal portion of the balloon tube with a silk thread with its open end extending to the midportion of the balloon. The other end of the tube is connected to the stem of a small glass funnel which has been heated and drawn out to fit the tube. Within the funnel, a pipette with a tapering end fitted with a rubber cuff fits tightly into the opening of the funnel stem. A rubber tube and clamp completes the pipette, so that any desired fluid may be retained therein until the desired moment, when by releasing the clamp it may be permitted to flow slowly through the tube and

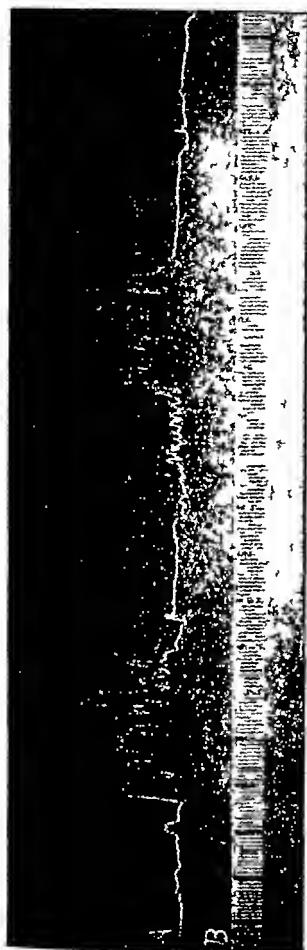


Fig. 4.—Decerebrate pigeon. A, periodicity of peristalsis; B, water manometer. Note the periodicity of the crop peristalsis. Note the periodicity of the crop peristalsis. Note the periodicity of the crop peristalsis.

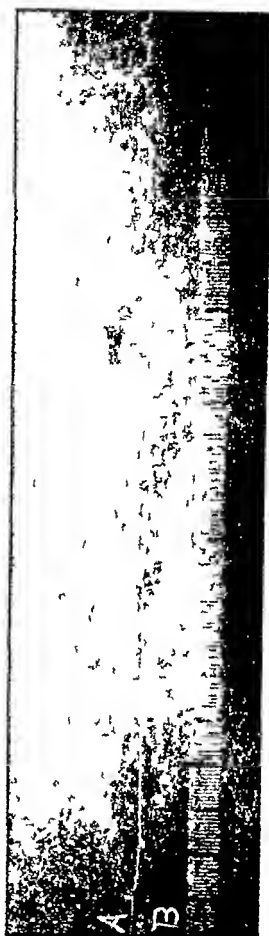


Fig. 5.—Decerebrate pigeon. A, digestive peristalsis of crop thirty to forty five minutes after feeding; B, time in five second intervals. Water manometer. Food in crop. B time.

directly into the crop (Fig. 3). In the case of water there appears to be some quantitative relation between the volume of fluid introduced and the relaxation of tonus. Thus 2 to 4 cc of water does not always produce this inhibitory action while 8 to 12 cc is effective (Fig. 6). More recently it has been found that very small amounts of food given to monkeys do not lead usually to gastric inhibition, while larger quantities prove effective.⁸ In

cases where a study of inhibition is desired a large fistula should be made in the crop and a rubber tube one-half inch in diameter inserted

It is therefore possible to utilize to advantage the decerebrate pigeon for a study of peristalsis for removal of the cerebral hemispheres does not materially affect the peristaltic movements of the crop This animal is even more suitable for use in the general student laboratory for such a study than the bullfrog, since decerebration transforms the nervous active bird into a stupid, lethargic creature which reacts only when stimulated Hence, the bird in addition to exhibiting the classical effects of decerebration may be satisfactorily utilized even under the disturbing influences of the student



Fig 6—Decerebrate pigeon A, hunger contractions of crop thirty two hours after feeding crop empty B signal magnet At X 8 cc water at room temperature was introduced directly into crop Note the abrupt termination of the period of hunger activity inhibition Water manometer (Tracing reduced about one-half)

laboratory for a study of peristalsis Furthermore, bullfrogs are difficult to obtain during certain seasons of the year while pigeons are more readily obtainable and this coupled with the combined studies of decerebration and gastric peristalsis on one and the same animal leads to an economy of animal material and a financial saving

REFERENCES

- ¹Patterson JOUR LAB CLIN MED, 1920, v, 674
- ²Rogers Am Jour Physiol, 1916, xli, 555
- ³Rossi Arch d Fisiol, 1904, ii, 376
- ⁴Doyon Arch de Physiol, 1894, 888, Arch de physiol norm et path, v Série, 1894, T vi, Année 26, 869 and 887
- ⁵Cannon Am Jour Physiol, 1911, xxix, 250
- ⁶Bronn Klassen und Ordnungen des Thier Reichs, vi, Vogel, Leipzig, 1891
- ⁷Kato Arch f des ges Physiol, 1914, cliv, 6
- ⁸Patterson Experiments not yet published

A SIMPLIFIED METHOD FOR THE PREPARATION OF SPECIAL LIQUID BLOOD MEDIA BY FILTRATION*

By A J SULLE PH D BERKELEY CALIF

DURING the past two years I have used large quantities of hemoglobin media for metabolism experiments and have found it exceedingly difficult to obtain sterile filtrates. The trouble does not rest so much in obtaining a sterile filtrate in the receptacle but in the manipulations of transferring it from the flask to sterile containers. When a quantity of about 200 cc of filtered medium is distributed the environment must be absolutely dust free, or otherwise, contaminations are the rule.

The preparation of hemoglobin mediums is always a time consuming process. It was usually handled as follows. An animal was exsanguinated, the blood laked and the hemoglobin solution mixed with the medium. The whole was centrifuged before passing it through a sterile Mandler filter. A contamination of the filtered medium always meant a considerable loss of time and material.

In order to overcome the disadvantages of the usual methods of filtration the following method has been adopted. Two Pyrex filter flasks *A* and *B* of 250 cc capacity each are connected to a T tube *C* by means of two pieces of pressure tubing of one eighth inch bore and one eighth inch wall. This T tube has an outer diameter of one fourth inch and a slight enlargement near the opening of each arm. The two lower enlargements are to hold the rubber tubing tightly while the upper one is to hold a tight packing of cotton. Both flasks take a No. 7 rubber stopper.

A glass tube *F*, three eighths of an inch outside diameter, projects through stopper *D* containing a plug of cotton and gauze in the upper end. Stopper *D* is now inserted into the flask and a layer of cotton wrapped around the mouth to prevent contamination. This cotton is covered with a piece of paper and fastened by means of a rubber band. A piece of paper is placed over the opening of tube *F*. This is also fastened with a rubber band. The preparation of flask *B* is thus complete.

Stopper *E* contains two glass tubes the larger one one fourth inch outside diameter and the smaller one three sixteenths inch outside diameter. The smaller tube contains an enlargement at the upper end to hold a tight packing of cotton. A rubber tube *L* is slipped over this enlargement the open end is closed by means of a piece of solid glass tubing. Stopper *E* is now inserted into the flask and covered with cotton and paper similar to stopper *D*. A piece of pressure tubing *G* projects from the upper end of the larger tube. The opening *H* is also covered with paper and fastened with a rubber band.

*From the Division of Bacteriology, University of California Medical School, Berkeley, California.

This completes the preparation of flask *A*. Both are sterilized in the auto-clave, after which they are ready for use.

The filter candles are wrapped in paper and sterilized as customary. The wrapping around a sterile candle *I* and the paper covering *H* are both removed, the openings flamed and the candle inserted into the pressure tubing. The mantle of the candle is fastened to a ringstand.

When the setup is complete the medium is placed on the filter and the pump connected with the apparatus through tube opening *C*. A screw clamp is placed at *K* in order that no air will be drawn through at *F*. After the stopper *E* is forced tightly into the neck of the flask the pump is turned on. The filtered medium collects in *A*.

When the filtration is complete, the pump is shut off, the screw clamp at *K* is removed and placed at *G*. The candle is then disconnected from the

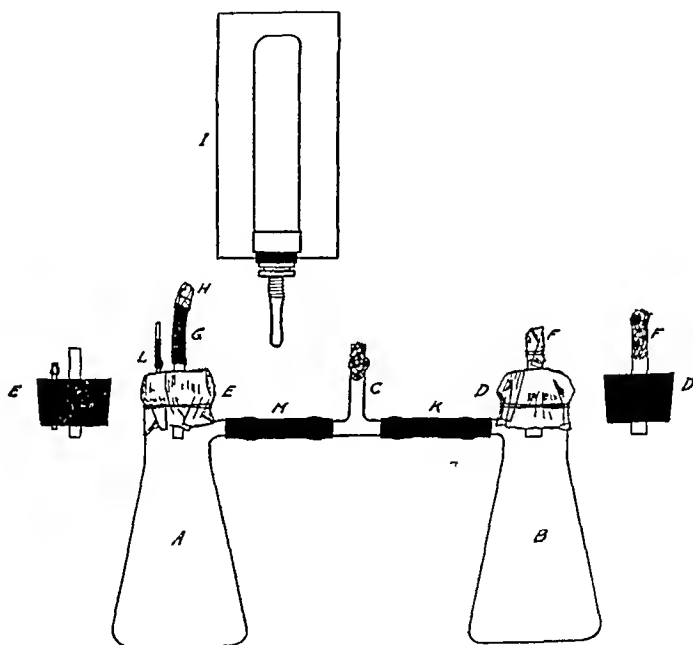


Fig 1

flask. The glass rod and rubber tube *L* are removed to let air into the flask. By raising flask *A* one-half of the medium is poured into flask *B*. Screw clamps are placed at *M* and *K*, after which the T-tube connecting the two flasks is removed. Flask *A* serves as a control while flask *B* is inoculated through the tube at *F*.

With such an arrangement only two sources for contamination must be considered: (1) The manipulations while connecting the candle to the pressure tube and (2) while making the inoculations through *F*. Since these openings are very small the chances for contaminations are slight. Over fifty samples of media have been filtered by means of this apparatus with only one contamination, while with the ordinary method they were the rule rather than the exception.

AN IMPROVED METAL CANNULA*

By R. R. DURANT, M.Sc., COLUMBUS OHIO

THE cannula is an instrument of general and varied use in physiologic experimentation. A large number varying more or less in style and material are in use. Each has its good and bad qualities which fact has led the writer to develop the use of the instrument described here. To be satisfactory a cannula should be cheap and durable, easy to handle and interfere as little as possible with the natural phenomena being observed. Credit for the original suggestion is due Dr. E. C. Albritton formerly a Fellow in this department.

The instrument is made from a Luer hypodermic needle of desirable size and length, having the end smoothed and a small knob of solder placed on one side (Fig. 1).

In this laboratory cannulae of this type are used wherever permanent connection with a vessel is desired, the trachea of course excepted.

For recording of blood pressure by the direct method a needle of approximately 1 cm. in length is used. After being tied into the artery it is con-

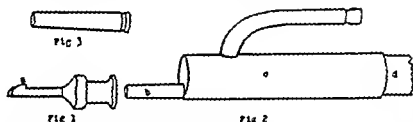


Fig. 1—Hypodermic needle shortened according to need, tip beveled and smoothed and a drop of solder (a) placed near tip.

Fig. 2—Connection for recording blood pressure. (b) Adapter having a taper of 15 to fit the cannula, turned from brass tubing (not nicked) and soldered to (c) reservoir for mixing anticoagulant with the blood, made of brass tubing 10 mm in diameter and approximately 5 cm. long. (d) Block tin pipe having inside diameter 3/16 in. and 3/64 in. wall, approximately one foot long, supported by means of a burette clamp on the stand bearing the manometer and signal magnets. (e) Brass tube 6 mm in diameter to receive the rubber tube from the pressure bottle. (c) and (e) may be nicked.

Fig. 3—Adapter similar to that in Fig. 2 (b) but slightly longer and grooved near the end as illustrated for securing the rubber tube connecting with pressure bottle.

nected with the manometer by means of a tapered adapter (Fig. 2), similar to that at the outlet of a syringe. A slight twist secures it against coming off even when subjected to the greatest arterial pressure. A twist is sufficient for removal during or at close of the experiment. The size required varies with the size of the animal. For large dogs a gauge 12 needle has a lumen of sufficient size that the actual blood pressure will not be reduced. A gauge 15 needle accommodates the artery of an average sized cat or rabbit. For small animals, rat, guinea pig or kitten the smaller needles—gauge 24-18—will suffice. Arterial cannulae of this type have a wall of minimum thickness and consequently a relatively large lumen. The danger of clotting in the cannula is correspondingly reduced. Using a gauge 15 needle a continuous blood pressure

*From the Department of Physiology, College of Medicine, Ohio State University.
Received for publication January 19, 1911.

record can be made for a maximum allowable period of time (one to three hours) without clotting. Records lasting ten to twenty minutes can be obtained without clotting using a gauge 20 needle. It is obvious that the use of such a cannula eliminates the necessity for various sizes of tubing, etc. The blood pressures of an 80 gm rat and 15 kg dog have been recorded with the same apparatus by simply changing the cannulae. That the use of the smaller cannulae gives fairly accurate results is shown by simultaneously recording the pressure in two paired arteries equidistant from the heart, using gauge 20 and 15 needles respectively. The pressure in the first artery is found to be only 5 mm Hg lower than that in the second.

Cannulae of the same type can be used for intravenous injection or for cannulating ducts. A syringe can be used for the reservoir and source of pressure or a hub (Fig 3) may make connection with a pressure bottle. A gauge 22 needle makes an easily applied cannula for use in recording urine flow from the ureter, even in very small animals, and for cannulating the ducts of submaxillary and pancreatic glands. Cannulae of this type have the following qualities for which it is believed their use will be found to relieve some of the difficulties of laboratory work.

- 1 Expense The cost is hardly more than that of the needle.

- 2 Durability Ordinary use is never strenuous enough to break such cannulae.

- 3 Ease of Use The cumbersomeness of glass T cannulae is eliminated, slipping out of the vessel is nearly impossible, small and delicate vessels can be cannulated with little danger of tearing, connection with other apparatus is simple.

- 4 The relatively large lumen affords free passage of fluids and therefore reduces error such as is unavoidable in the use of small glass cannulae.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE MD ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

MENINGITIS The Chemotherapy and Serum Therapy of Pneumococcus and Streptococcus Meningitis Kolmer J A Arch Otolaryngology June 1926 III, 481

Polyvalent antistreptococcus serum administered by intramuscular, intravenous, intracisternal and intraventricular injection has failed to influence appreciably the cause and mortality of experimental hemolytic streptococcus meningitis of dogs

Type I antipneumococcus serum administered by intramuscular, intravenous intracisternal and intraventricular injection has failed to influence appreciably the course and mortality of experimental type I pneumococcus meningitis of dogs.

The intravenous, intraventricular and intracisternal injection of antipneumococcus antibody solution has exerted a slight degree of curative activity in the treatment of pneumococcus meningitis

Lavage of one or both of the lateral ventricles to the cisterna magna has proved effective in the treatment of severe experiment streptococcus and pneumococcus meningitis of dogs This may be combined with the administration of antibody solution by subtheal injection in the treatment of pneumococcus meningitis or by the injection of gentian violet in the treatment of streptococcus meningitis

NEOPLASMS The Etiology of Malignant Neoplasms London, J McCormack J M and Howard N J Canadian Med Assn Jour May 1926 XVI No 5, p 523

The authors believe the cause of neoplasms to be a Gram positive pleomorphic organism which they have isolated from fresh human neoplastic tissues and from neoplasms in rats and chickens and from the blood of the hosts In one of its phases this organism is filterable and invisible under the microscope

It is admitted that infection does not usually take place unless the defences of the cell have been broken by some preceding condition which lowers resistance to infection

The paper is a summary of previous publications and studies to date

LEUCEMIA Leucemia and the Central Nervous System, Fried B M Arch Path and Lab Med, July, 1926 I: No 1, p 23

A case of leucemic lymphadenosis is reported and thirty cases of leucemic lymphadenosis and myeladenosis from the literature involving the central nervous system, are reviewed

In the author's case a patient with a subacute leucemic lymphadenosis died of apoplexy, and at necropsy numerous lymphomas and hemorrhages were found in the brain Grave degenerative changes were found in the interstitial parenchymatous and mesenchymal elements of the brain, more pronounced in the vicinity of the extravasated blood and also around the lymphomas. These changes are not regarded as peculiar to leucemia, but are ascribed to circulatory disturbances in the brain due to hemorrhages and accumulated masses of lymphoid elements (similar lesions of a minor degree occur in primary and secondary malignancy of the brain)

In the thirty cases gathered from the literature lesions of the nervous system have been reported in the hemispheres in twelve, in the cranial nerves in eight and in the cord in eleven cases, in eight of which spinal degenerations were observed in the absence of lymphomas, in many respects resembling those observed in pernicious anemia

Hemorrhages in leucemia are primarily due to vascular lesions produced by (1) stasis in the capillaries and "thrombosis" of the vessels by lymphoid cells, (2) the invasion of the vessel walls by the lymphoid cells with dissociation of the vascular coat and (3) the hypotonic "toxin" which circulates in leucemic blood

The occurrence of lymphomas in organs or structures in which lymphoid tissue is normally absent (as in the brain) is due to the passage of the vascular wall by lymphoid cells, with the ultimate formation of large nodules which occasionally grow as autochthonous units. This phenomenon is defined as "colonization" by lymphoid elements and is to be distinguished by metastases in malignancy.

PERNICIOUS ANEMIA Familial Pernicious Anemia, Dorst, S E Am Jour Med Sc, August, 1926, CLXIII, No 2, p 173

The recent observation of a patient with pernicious anemia, five other members of whose family had died of it or had had the same disease, suggested an investigation of the other members of the family group, which disclosed the fact that four more had either marked hypochlorhydria or complete achlorhydria.

Dorst feels certain that if it can be shown that achlorhydria is a predominating characteristic in families, one or more members of which have pernicious anemia, then there would seem to be no question but that "congenital" achylia gastrica is undoubtedly the hereditary factor in cases of familial Addison's anemia, and that gastric analysis in the members of the immediate family of a patient with pernicious anemia would show evidence of achylia gastrica, of congenital or hereditary origin.

A detailed account is given of the cases mentioned above and Dorst concluded with these queries: Can the development of pernicious anemia be prevented by the administration of hydrochloric acid to patients who have an achlorhydria and a familial history of the disease? Is similar therapy of value in the early stages of the disease? These questions cannot be answered until our clinical cases have been followed for the next five or ten years, or without an exhaustive experimental investigation.

LABORATORY TECHNIC

TISSUE TECHNIC A New Method of Mounting Fixed Frozen Sections, Campbell, L D Arch Path and Lab Med, June, 1926, I, No 6, p 916

It is easier to work over a black table. The steps are as follows:

1. Previously fixed tissues are cut with a freezing microtome in sections 7 to 12 microns thick, which are placed in a large glass dish containing water.
2. A section is lifted out with a glass rod with the section wrapped smoothly around the rod near its end, and held in 95 per cent alcohol for two or three seconds.
3. The section is returned to a large, deep staining dish of water (about 7 cm deep), unrolling the section from the rod on top of the water. In most cases it will float on the surface and be perfectly smooth.
4. A glass slide is brought against one edge of the floating section, holding the slide at almost right angles to the surface, and is lifted out. The section will adhere to the slide. If it has not been held in alcohol sufficiently long, or if too much time elapses before mounting after returning to the water, it will sink beneath the surface and cannot be readily mounted. If a fold should occur, the slide should be held on the edge and dipped carefully up and down in the water (not immersing the entire section), and the fold will easily be removed.

5. The mount is completed as usual by dropping on 95 per cent alcohol to dehydrate. It is blotted with a smooth folded cloth, and very thin celloidin is dropped on the section, holding the slide almost perpendicular to the table.

When the celloidin begins to solidify it is to be stained as desired.

UREA IN SALIVA A Quantitative Method for the Determination of the Combined Urea and Ammonia Nitrogen of Saliva Schultz F W and Ziegler M R Am Jour Dis Child, April, 1920, *xxxi*, 520

Ten cc saliva collected without the use of a stimulant is immediately shaken for five minutes with 2 gm of nitrogen free kaolin and filtered. The kaolin is prepared in quantity from Merck's kaolin by washing twice with 2 per cent acetic acid, four or five times with distilled water, filtering and drying. If the filtrate is turbid, the process is repeated. To 1 cc of the saliva filtrate in a 75 cc pyrex test tube add 4 cc of distilled water, 2 drops of Folin's pyrophosphate buffer mixture and 1 cc of his urease solution. This is heated in a water bath, from 40 to 55 C for five minutes. The ammonia, including that formed from the urea, is removed by aeration after the addition to the digestion mixture of a little liquid petroleum and 2 cc of 10 per cent sodium hydroxide. It is collected in a test tube graduated at 25 cc and containing 2 cc of 0.05 normal hydrochloric acid diluted with about 10 cc of water. After fifteen minutes aeration dilute the contents of the receiver to 20 cc., add 25 cc of Nessler's solution, make up to 25 cc and compare in the colorimeter with a standard containing 0.3 mg of nitrogen (in the form of ammonium sulphate) and 10 cc of Nessler's solution in a 100 cc flask.

Calculation

$$\frac{\text{Reading of standard in mm}}{\text{Reading of unknown in mm}} \times 0.3 \times \frac{1}{4} \times \frac{100}{1} = \text{mg of ammonia and urea N per 100 cc of saliva}$$

Samples of blood and saliva were collected simultaneously. The determination of blood urea nitrogen was carried out according to the directions of Folin and Wu using the aeration method.

AMEBIC INFECTION Detection of Amebae in Cases of Chronic Systemic Amebic Infection, Albert H. Am Jour Pub Health April 1926

Directions for the collection of specimens of feces to be examined for amebae

1 Give patient eighteen 5 grain tablets of Glycotauro (bile salts) with instructions to take one tablet two hours after each meal for six days.

2 Give patient six small wide mouth well stoppered bottles with instructions to collect a small portion of feces (about the size of a small marble) each day for six successive days beginning the day after starting on the bile salts.

3 Send specimens to the laboratory as soon as possible. If the laboratory cannot be reached within a day, pour a small amount (about one fourth volume of feces) of 10 per cent formalin (one part of the commercial formalin to ten parts of water) over the feces.

The laboratory examination consists of a search chiefly for the cyst forms of the parasite, since these are, as a rule, very much more numerous than the motile vegetative forms. This is especially true of chronic amebiasis. In cases which present a rather definite clinical picture of amebiasis and cysts have not been found on repeated examination, it is advisable to make an examination for the vegetative forms also. Specimens to be examined for such should be kept at body temperature by means of a vacuum bottle and a warm stage.

Technic of Examination

Two methods are used: (1) a direct smear of fresh fecal matter stained with a modified Donaldson's iodine eosin, and (2) a fixed preparation stained with Haidenhain's iron hematoxylin.

A drop of normal salt solution and one of iodine eosin stain are placed close together on a slide but not touching. A round applicator stick or a toothpick is smeared with the feces, rolled in the drop of normal salt then in the drop of iodine eosin. A single cover slip is placed on both drops, half the material under it being stained and the other half unstained. Examine the unstained portion first for living flagellates and active amebae. In the stained

portion the protozoan cysts stand out as bright spherules against the pink background and soon become tinged with the iodine to varying tones of yellow, with the nuclei becoming clearly defined as the iodine penetrates. If glycogen is present in the cysts, it becomes light or dark brown in color.

Iodine eosin stain

- a Saturated aqueous solution eosin in normal salt----- 2 parts
- b Five per cent potassium iodide in normal salt
solution saturated with iodine----- 1 part
- c Normal salt solution----- 2 parts

The proportion of iodine solution used may be modified to advantage by adding a slight excess of that given in the formula if the nuclei do not appear after a few moments' application of the stain. The stain should be made up each day from the stock ingredients.

2 For fixed preparations a smear is made on a slide which has been previously thoroughly cleaned in alcohol-ether and flamed. If the fecal material is too dry, moisten slightly with normal salt, make a thin smear with the applicator stick or the flat side of a toothpick or by using the edge of another slide or a cover slip, and immerse directly in fixing fluid *without allowing the slide to become dry*.

Fixing and Staining Methods Used

- 1 Schaudinn's fluid (even if previously fixed by formalin)----- 5 min
- 2 Seventy per cent alcohol tinged with Gram's iodine----- 5 min
- 3 Seventy per cent alcohol----- 5 min
- 4 Fifty per cent alcohol----- 5 min
- 5 Tap water ----- 2 min
- 6 Two per cent iron alum aqueous solution----- 5 min to 12 hrs.
Or 2 per cent iron alum aqueous solution heated to 30° C (never higher)----- 10 min
- 7 Tap water—running ----- 5 min
- 8 Five tenths per cent hematoxylin aqueous solution (Hardenham's)----- 12 to 18 hrs.
Or 0.5 per cent hematoxylin aqueous solution heated to 30° C (never higher)
----- 10 min to 1 hr
- 9 Tap water rinse
- 10 Differentiate in 1 per cent iron alum with careful watching under the microscope
----- 3 to 30 min
- 11 Wash in running water----- 10 min
- 12 Fifty per cent alcohol----- 5 min
- 13 Seventy per cent alcohol----- 5 min
- 14 Ninety per cent alcohol----- 5 min
- 15 One hundred per cent alcohol----- 5 min
- 16 Xylol ----- 5 min
- 17 Mount in balsam—cover

Schaudinn's fluid—two parts saturated aqueous HgCl_2 in normal salt, one part absolute or 95 per cent alcohol. Add 4 c.c. glacial acetic acid to 96 c.c. of the mixture on using.

If a quick method is desired, the same outline can be followed with a shortening of the time of application of the iron alum and the hematoxylin. The slide is taken from the water, flooded with or in alum by a pipette and held over a flame or placed upon a heated plate for about five minutes, or until it begins to steam. Wash in water and treat in the same way with the hematoxylin, continuing the application by heat until a metallic scum appears on the top of the fluid on the cover slide. Differentiate in iron alum. Care must be taken throughout the entire process to avoid drying of the smear. Use American hematoxylin, standardized white crystals only. Use only violet crystals of iron alum, reject yellowish powder.

COLLOIDAL GOLD The Correction of Colloidal Gold Solutions as Applied to the Lange Reaction Novich N Arch Neurol and Psychiatry April, 1926, xv, No 4, p 471

METHOD OF PROCEDURE

NUMBER OF TUBE	1	2	3	4	5	6	7	8	9	10	11	CONTROL
Twentieth normal sodium hydroxide or twentieth normal hydrochloric acid (as required) cubic centimeters	0.05	0.075	0.1	0.15	0.2	0.25	0.275	0.3	0.35	0.375	1.40	None
Colloidal gold solution (as prepared) cubic centimeters	5	5	5	5	5	5	5	5	5	5	5	5
1 per cent sodium chloride solution, cubic centimeters	17	17	17	17	17	17	17	17	17	17	17	17

The series of tubes thus set up are set aside at room temperature protected from light, and read at the end of one hour. The tube showing complete precipitation and containing the least amount of acid or alkali is taken as the correction point for the solution. The amount needed for the whole is calculated and added to the prepared solution. The control tube serves as a preliminary indicator and shows whether acid or alkali is needed for correction. It is not advisable to use an acid or alkali solution of higher normality because it would affect the strength of the sodium chloride solution used as an indicator an important factor.

It should be remembered that a colloidal gold solution at best, when tested with a clinically pathologic cerebrospinal fluid does not precipitate in the first or second tubes or high concentration at once, but the higher dilutions (1:160 to 1:320) take precedence and precipitate almost immediately. This is probably due to the fact that the high concentration of a fluid that has a high protein content (globulins) brings about a condition of surface tension unfavorable for immediate precipitation. It should also be remembered that the method of titrations as outlined, cannot be used for solutions grossly improper because of noncompliance with technical requirements.

Conclusions

1 In the preparation of a good colloidal gold solution certain technical difficulties, at best unavoidable, are frequently encountered. Many solutions are discarded as unfit though prepared with great care.

2 The primary cause of unsuitable solutions is the reaction of the final product.

3 The old method of titration using alizarin as indicator is not entirely satisfactory. It does not visibly "indicate" because of the primary color of the solution under titration.

4 A method of titration using 1 per cent sodium chloride solution to the extent of 17 c.c. as an indicator is suggested. This salt is an electrolyte and a precipitant of colloidal solutions, and serves as an accurate and highly satisfactory indicator of the reaction state of a prepared colloidal gold solution.

SPIROCHETA PALLIDA Experiments on the Purification of Cultures of Spirocheta Pallida by Chemical Methods Weiss D and Weiss C Jour Infect Dis, April 1926 xxviii, No 4 p 281

Experiments were undertaken to determine the selective inhibitory action of various germicidal substances on the growth of bacteria which may contaminate cultures of Spirocheta pallida.

The following chemicals are satisfactory (in the dilution and time of exposure stated) for the purpose of destroying *B. coli* as well as *Staphylococcus aureus* without affecting the viability of the reproductive power of *Spirocheta pallida*, Selenium oxychloride or trichloro-ol (in a 1 100 dilution, to be used for one minute), trichloroacetic acid (1 100 for fifteen minutes) or formaldehyde (1 20 for five minutes)

When it is desired to destroy staphylococci alone, a larger variety of chemicals may be employed. Gentian violet, acid fuchsin, mercurochrome, mercuraphen, methylene blue, monarsone, neoarsphenamine, atoxyl, acid arsphenamine, antiformin, Lugol's solution (iodine), ethylhydrocuprein hydrochloride or neosilvol.

TUBERCULOSIS Culture of Tubercles in the Diagnosis of Tuberculosis, Hohn, J. Munchen med. Wchnschr., April 19, 1926, LVIII, 609

To 10 cc of material to be cultured add 1 to 2 cc of sulphuric acid 10 per cent in a test tube and allow to stand thirty minutes. The tubes are shaken from time to time. Centrifuge for five minutes and inoculate the sediment on three to four egg tubes.

GONORRHEA The Diagnosis of Gonorrhea by Culture, Gradwohl, R. B. H. Jour. Am. Med. Assn., July 24, 1926, LXVIII, 242

The following media has given good results in the author's hands in primary cultures.

Five hundred grams of ground lean beef is infused in 500 cc of distilled water and allowed to stand in the ice box over night. The following morning, 30 gm of agar is dissolved by boiling in 500 cc of distilled water. The agar is allowed to cool to between 60° and 70° C, and the meat infusion is immediately mixed with the agar. This mixture is again heated until the meat is thoroughly coagulated. It is then filtered through glass wool packed in the stem of a glass funnel. This filtrate will appear slightly cloudy at this time. In the filtrate is dissolved 1 per cent peptone and 0.5 per cent sodium chloride.

The medium is adjusted to P_H 7.6 to 7.8, allowed to cool to 60° C and the white of an egg thoroughly mixed in. It is next brought to a boil and again filtered through glass wool. If the flask and filtering funnel are placed in an Arnold sterilizer, the filtration is hastened and a crystal clear agar results.

To this medium is added 1 per cent chemically pure levulose (made from molasses), and sufficient 0.5 per cent aqueous bromocresol purple indicator to color the medium a rich purple. The whole is then autoclaved at five pounds pressure for forty five minutes. When the medium has cooled to between 60° and 70° C one part of sterile ascitic fluid, guinea pig serum or human serum is added to three parts of medium, and the plates are poured to a depth of about one eighth inch. The plates may then be stored in the ice box until ready for use. In inoculating plates, best results will be obtained if straight line streaks are made with a platinum loop, thus preventing confluence of colonies.

In the cover of the Petri dish is inserted a piece of common blotting paper saturated with hydrogen peroxide. The plate is then inverted and incubated over night, and the next morning the suspicious colonies are fished and stained by Gram's method.

All colonies exhibiting a zone of yellow (showing acid production from levulose) are disregarded. All colonies showing an opaque whitish luster similar to *Staphylococcus albus* are disregarded. Typical colonies of *Neisseria gonorrhea* can be identified by the low power objective of the microscope, the 9x eyepiece or even by the naked eye after practice. The colonies in eighteen hours are about the size of a pin head, round translucent, finely granular, and with a pearly opalescence by transmitted light—the latter characteristic being always striking. The colonies also are mucoid, adhering to the medium, though emulsifying in water fairly easily. On staining, the individual organisms are moderately large, decolorize very quickly in 95 per cent alcohol, and exhibit the typical appearance of small groups and single organisms.

After eighteen or twenty four hours, the Gram stain shows the colony to contain considerable numbers of autolyzed organisms—a very important diagnostic point. Also after this period the individual colonies enlarge to considerable size, and show many supergrowths,

radial striations, lobated margin and concentric 'oil drop' appearance. N gonorrhea may be distinguished from *Micrococcus catarrhalis* by its failure to grow on common infusion agar and its tendency toward early autolysis.

TUBERCULIN Active Principles of Tuberculin Prepared from Nonprotein Substrates
Eberson, F. *Am Rev of Tub*, May 1936 **Vol** 5 p 454

A report of the author's studies of tuberculin prepared from nonprotein synthetic media.

The medium was the following:

Ammonium succinate	- - -	0.5 gm
Dipotassium phosphate	-	0.5 gm
Magnesium sulphate		0.25 gm
Calcium chloride		0.135 gm
Distilled water	- -	100.0 cc

To one portion of this substrate 2 per cent glycerine was added. After the tubercle bacilli had been growing on this medium for five to six weeks the tuberculin was prepared according to the usual method by filtration and concentration to one tenth of the original volume. The growth and staining properties of tubercle bacilli were modified by this synthetic medium. In the glycerinated portion the microorganisms grew more luxuriantly than in the portion to which no glycerine had been added. In the former half of the microorganisms lost their original acid fast characteristic while all of them lost it in the glycerine free medium.

From cultures grown on this medium a tuberculin was prepared by fractional alcohol ether precipitation. The final ether insoluble fraction represented less than 0.5 per cent by weight of the original tuberculin contained approximately 4.4 gm of active substance per cc and gave none of the usual tests for protein.

The results of the study may be thus summarized:

Three fractions have been derived by chemical methods for tuberculin prepared with synthetic nonprotein media. They represent alcohol insoluble, ether insoluble and ether soluble substances, and comprise, respectively 4.6 per cent, 2.3 per cent and approximately 0.5 per cent by weight of the original tuberculin. The ether soluble fraction is gummy and fatty or waxy, and gives none of the tests for protein.

In the tuberculous guinea pig the potency and specificity of these tuberculin fractions have been demonstrated as early as three days after inoculation of the animal with B. tuberculosis.

It has been shown for the first time that small amounts of tuberculin fractions prepared from protein free synthetic substrates are capable of sensitizing normal nontuberculous guinea pigs. In these animals typical skin reactions can be elicited subsequently by intracutaneous injections of minute amounts of homologous as well as heterologous fractions and of unfractionated tuberculin from which such fractions have been prepared.

Clinical trial of these fractions in juvenile patients has demonstrated for the first time that the substances have diagnostic value. Positive skin tests were perfectly correlated with positive clinical and laboratory findings of tuberculous infection. Such was not the case for ordinary old tuberculin in over 20 per cent of young patients in a group of 150 who were studied routinely. In a number of instances the observations suggested that the reactions might be correlated with the degree of activity or with arrested tuberculous infection.

The active substance contained in a dose used for intracutaneous tests in patients was calculated as follows: alcohol insoluble fraction, 0.005 mg; ether insoluble fraction 0.0025 mg; ether soluble fraction 0.0005 mg.

The present studies point to a method which makes an accurately standardized tuberculin available for routine clinical work. It is believed that the active principle of tuberculin can best be isolated by this or by a similar method.

The observations on sensitization suggest that these may be applied to studies of immunity and, by extension to therapeutic studies in the experimental animal. Such work, now in its third year of progress will be reported in the future.

GRANULOMA Studies in Coccidioidal Granuloma, III Mode of Infection, Ahlfeldt, F E
Arch Path and Lab Med, August, 1926, 11, No 2, 206

Experiments dealing with the mode of infection were attempted by rubbing one platinum loopful of the dry mold over an abraded area of the skin, over the mucous membrane of the nose, over the mucous membrane of the mouth and by suspending a platinum loopful in salt solution and injecting it into the trachea through the skin in rabbits and guinea pigs Also, experiments dealing with the natural mode of infection were attempted by feeding guinea pigs with lettuce contaminated with culture and exposing guinea pigs to air contaminated with broth culture

Rabbits were more resistant than guinea pigs All the guinea pigs died spontaneously in from three to four weeks They maintained their health until the tenth day and then lost in weight quickly There was a characteristic drop in weight At necropsy, some of the pigs showed minute nodules in the lungs and liver, some enlarged mesenteric and cervical lymph nodes, some suppurative periorchitis Microscopically, there were small areas of peribronchial inflammation, edema and hemorrhage in the lung The liver showed some cloudy swelling, the spleen follicular hyperplasia, the stomach and intestines were normal, the kidney showed a mild acute interstitial nephritis, the lymph glands some hemorrhage, and the brain showed edema The organism was found in the lung, liver, intestine, lymph gland and spleen

Guinea pigs fed coccidioides culture on lettuce showed no gross lesions and insignificant microscopic lesions, few adult forms and shells in the intestines and some adult forms in the pharyngeal lymphatics, they lived longer than pigs that breathed culture The animals that breathed the culture showed deep congestion or hemorrhages in the lungs, and fluid in the pleura, but no more of the adult forms could be found in the lungs than were discoverable in the tissues of the feeding pigs These differences suggest greater potency of infection by breathing contaminated air than by eating infected lettuce

As the animals were infected by using the dry mold through the skin, the trachea and the mucous membrane of the mouth, and by exposure to contaminated food and air, it is probable that coccidioidal granuloma may be transmitted through the skin, as well as the respiratory and gastrointestinal tracts

CARCINOMA The Quantitative Determination of Albumin by Tannin and Its Use in the
Diagnosis of Cancer, Wigand, R Munchen med Wehnschr, March 26, 1926, lxxii,
521

Twenty four test tubes were filled with the same serum diluted in physiologic salt solution in descending geometric progression, so that the first contained a 1:10 dilution, the second a 1:20, the third a 1:40 solution, etc To each of the tubes, containing 2 c.c. of the dilution, 1 per cent of a limpid, freshly prepared and filtered tannic acid was added After eight to twelve hours the precipitated albumin was read The slight veil which rose from the bottom of the higher dilutions was more clearly visible when 2 to 3 drops of a strongly diluted carbolfuchsin solution were added to the tannic acid The albumin in the first seven tubes settled in dense masses immediately after the addition of the precipitant, in the following 6 to 7 tubes the precipitation lasted several hours, in twenty four to forty eight hours even the weakest concentrations showed the flocculation In this way 0.000001 gm. was determined This is not possible by any other method

A series of tests shows that cachectic individuals do not react positively, that the reaction fails in scirrhus carcinoma and after surgical interventions Medullary carcinomata are the most frequent to be diagnosed The serum of pregnant women in the first four months is never positive Syphilis and tuberculosis seem to have equal results

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*The Pathology and Treatment of Diabetes Mellitus**

THE first edition of this volume was reviewed in Volume x page 590 of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE. This edition has been brought up to date and has many new observations on insulin and on dietary treatment with insulin. All who make any pretense of interest in the problem of diabetes should possess this work.

The Medical Department of the United States Army in the World War Field Operations†

VOLUME EIGHT of the history of the Medical Department of the United States Army in the World War is a description of the organization and the activities of the Medical Department of the American Expeditionary Forces from the arrival of the advance guard until the armistice, with brief histories of the individual combat divisions. The volume is profusely illustrated with U S Army official photographs of various medical organizations and institutions in the A E F in France, Italy, Siberia and elsewhere.

It becomes at once evident to the reader that the preparation of this volume has required a tremendous amount of painstaking care. While much of it is bare narrative the human element has not been left out.

The work will naturally find its greatest usefulness as a subject for critical study by army medical officers in this and other countries.

The Thyroid Gland‡

A SMALL volume containing an historical development of the goiter subject and a presentation of the contributions that have been made to our knowledge of etiology, pathology, and treatment in the various departments of the Mayo Clinic.

When we consider that up until 1861 barely a hundred operations had been reported on the thyroid gland, we realize how great has been the progress in the last half century. In 1874 only two surgeons in France, Italy, Great Britain and America had performed more than four lobectomies. In the United States and Canada only forty five operations for goiter were recorded up to 1883.

*The Pathology and Treatment of Diabetes Mellitus. By George Graham M A M D F R C P. Cloth. Illustrated. Pp 230. Price \$2.75. Humphrey Milford Oxford University Press.

†The Medical Department of the United States Army in the World War. Field Operations. Prepared under the direction of Maj Gen M W Ireland. The Surgeon General by Col Charles Lynch M C. Col Joseph H Ford M C. Lieut Col Frank W Weed M C. Cloth. Illustrated. Pp 1097. Government Printing Office Washington D C 1915.

‡The Thyroid Gland. By Charles H Mayo and Henry W Plummer. Cloth. Pp 83. The C V Mosby Company St Louis Mo 1926.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

By contrast, when Kocher died in 1917, five thousand cases of goiter had been operated on at his clinic. At the Mayo Clinic from 1912 to 1922 there were twenty thousand, one hundred and sixty one resections of the thyroid with six thousand four hundred and ninety three ligations of thyroid arteries.

Dr. Plummer presents an excellent summary of our knowledge of the function of the thyroid gland. He gives abundant evidence in substantiation of his theory that the symptoms of Graves' disease are due to a dysfunction rather than hyperfunction of the gland. In toxic adenoma we are dealing with an increased secretion of normal thyroxin and the treatment indicated is removal of those tissues which are secreting the excess. In exophthalmic goiter on the other hand there is both a hypersecretion of normal thyroxin and the formation of an abnormal incompletely iodized thyroxin. The latter is responsible for the symptoms of Graves' disease. Here the administration of iodine gives greatest benefit by enabling the thyroid to completely iodize the thyroxin molecule. He presents the record of one patient who, before thyroidectomy, had a basal metabolic rate ranging above plus eighty and since operation a rate of minus fourteen, but with the characteristic nervous phenomena and a progressive exophthalmos still persisting. When this patient is placed on Lugol's solution the nervous phenomena disappear, exophthalmos recedes, the basal metabolism drops to minus twenty-eight and edema, slow speech, etc., characteristic of myxedema appear within two weeks. When iodine is administered and in addition the basal metabolism is maintained at the average normal rate with thyroxin or thyroid extract, all evidence of disease disappears. Here we have a patient who, after operation has a thyroid deficiency requiring the administration of thyroid extract and at the same time is putting out an abnormal thyroxin which requires Lugol's solution for saturation.

Practical Dietetics in Health and Disease¹

HAVE you, in your experience, had a patient who has had occasion to receive treatment from two different gastroenterologists both of outstanding repute? And then has the task been yours of trying to help straighten him out of the hopeless mental tangle and dismay in which he finds himself after discovering that these two men both equally positive and dogmatic in their statements prescribed quite different diets and that each insists not infrequently that articles of food looked upon with favor by the other are nearly in the class of rank poison?

There is much faddism in dietetics today. Dietetic prescriptions are dogmatically drawn up and rigidly enforced often where there is no sound scientific basis.

Of course, this is often necessary for the purpose of making the patient adhere to a reasonable general dietary.

The volume under review presents a comprehensive list of appropriate diets for a great variety of disorders. The different diseases are arranged alphabetically, thus facilitating the physician's finding the appropriate dietary recipe once his diagnosis has been established. Most of the diets are of a dogmatic type but they are distinctly useful in that they are something which the physician can give to his patient and which the patient can follow without difficulty.

The author makes no attempt to provide a comprehensive discussion of the dietary principles in those diseases in which diet is of the greatest importance such as food allergy, nephritis, diabetes, pellagra, ulcer, pernicious anemia. Appropriate dietaries for individual instances of these diseases are included.

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS, MO JULY 1927

NO 10

Editor in Chief WARREN T VAUGHAN M D

Richmond Va

ASSOCIATE EDITORS

DENNIS E JACKSON M D	CINCINNATI
PAUL G WOOLLEY M D	LOS ANGELES
J J R MACLEOD M B	TOPONTO
W C MACCARTY M D	ROCHESTER, MINN
GERALD B WEBB M D	COLORADO SPRINGS
VICTOR C MYERS PH D	IOWA CITY
RUSSELL L HADEN M D	ROSEDALE KAS
JOHN A KOLMER M D	PHILADELPHIA
ROBERT A KILDUPPE M D	ATLANTIC CITY N J
A PARKER HITCHENS M D	MANILA P I
DEAN LEWIS M D	BALTIMORE
GEORGE R HERMANN M D	NEW ORLEANS
HAROLD D CAYLOR M D	ROCHESTER MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis Mo as Second Class Matter

EDITORIALS

Ring and Flocculation Tests in Tuberculosis

A CERTAIN amount of attention is being given at present to a number of serum preecipitation (flocculation and ring) tests which have been introduced in the hope of giving diagnostic and prognostic information in tuberculosis. The tests which have been employed most in this country are Daranyi's,¹ a flocculation test performed with alcohol salt solution which has been studied by many including Enderle,² Pinner³ and Baum and Schumann⁴ who have modified the technique somewhat with a view to making the test more quantitative, the tests of Larson and his associates,⁵ ⁶ ⁷ ⁸ ⁹ ¹⁰ ¹¹ ¹² one of which involves the use of an extract of tubercle bacilli the other a 0.2 per cent solution of trieresol in 0.85 per cent sodium chloride solution, and a semisecret commercial preparation known as 'Tubercumet,'¹² ¹³ ¹⁴ to which we shall revert later.

In Europe a greater variety of these tests have been reported. Setting aside earlier studies for the moment, the tests now appearing in the literature

are Daranyi's, Matefy's,^{15 16} 0.05 per cent aluminum sulphate, Lange and Heuer's,^{16, 17} a derivative of the simple distilled water test attributed to Klausner, distilled water and 1 per cent silver nitrate plus light, Mundel's,^{18 19} 18 to 19 per cent ammonium sulphate, Bonacorsi's^{19, 20} a derivative of Dold and Sachs-Georgi tests, and a test bearing the name of Fornet,²¹ which is apparently being exploited commercially. The studies of Veines and Prunell²² with 1.25 per cent resorcin, of Sachs and Oettingen with heat, alcohol, sodium chloride, and ammonium sulphate, of Frish and Starlinger with saturated chloride solution, and of Sachs and Klopstock²² with lecithin and calcium chloride, should also be mentioned.

It will be noted that in nearly all these tests where the composition of the preparation is given with scientific candor, the solution contains some substance which is known to precipitate globulin within suitable ranges of concentration. Wherever the biochemistry has been seriously investigated the precipitate is believed to be due to increased colloid lability, which in turn is associated with increased serum globulin, perhaps in combination with other physicochemical factors such as variations in the amount of lipoids present, and the hydrogen-ion concentration.

In 1910 Porter²² made a very interesting and significant study of precipitation tests. He tested sera from several hundred persons by the following method. Three solutions were prepared, (a) Bacillary emulsion diluted 1:50, made isotonic (0.85 per cent NaCl), and filtered through porcelain, (b) The same with the addition of 0.5 per cent phenol, (c) 0.5 phenol in 0.85 per cent salt solution, without bacillary extract. Each of these solutions was mixed with an equal amount of serum diluted 1:20, and incubated twelve hours at 37° C. Positive results, which varied from a slight sediment to a marked precipitate in suspension, were secured in about 85 per cent of all tuberculous sera, the percentage was lowest in very advanced cases. About 30 per cent of sera from nontuberculous persons gave positive results, but this group included many patients with other diseases. These figures compare pretty favorably with most of those reported in subsequent studies on precipitation tests, though in some of the literature cases with doubtful diagnoses have obviously been grouped according to the result of the test, thus giving a false impression of great accuracy. The most interesting point is that Porter got almost identical results with all three solutions employed, and concluded that for practical purposes the phenol-salt solution alone was satisfactory. He calls attention to the fact that Stoerck,²³ in the preceding year (1909) had noted the precipitation of tuberculous sera by phenol. This reaction is now familiar and quite certainly explained by globulin increase and increased "colloid lability." But the cause of the precipitation with the isotonic 1:50 bacillary extract remains a problem, and a very interesting one.

Calmette²⁴ in his encyclopedic "*L'Infection Bacillaire et la Tuberculose*" reviews previous work on precipitation tests including studies of his own with Massol, first reported in 1910. Calmette and Massol²⁵ concluded that there was no evidence of a specific reaction between the dilute tuberculin

used and any component of tuberculous serum. Precipitates sometimes resulted, but these precipitates contained no tuberculin, and did not reduce the strength of the tuberculin used. They observed similar precipitation on diluting the sera with five volumes of distilled water, and attributed the positive results with tuberculin to dilution of the sera, implying that the tuberculins were diluted with distilled water.

Calmette cites Porter's work but unfortunately quotes his percentages incorrectly, and does not mention the precipitation by phenol salt solution alone nor the important point that Porter's dilute tuberculin without phenol was isotonic, so that there can be no question in that case of a simple precipitation of globulin in hypotonic solution.

There is a large recent literature on the precipitation tests dealing chiefly with the percentage of accuracy in known clinical conditions. The figures vary with different tests and in different reports but there is pretty general agreement on the following points:

- 1 The percentage of positive tests is very low in health
- 2 The percentage of positive tests is high in active tuberculosis
- 3 Tuberculous persons giving negative tests are most of them either extremely sick or virtually free from symptoms. Agreement on this point, however, is not complete
- 4 Any of the tests may be positive in a variety of conditions other than tuberculosis. They seem to be commonly positive in acute respiratory infections
- 5 A repeatedly positive test in the absence of other infectious disease may be of assistance in confirming a doubtful diagnosis of tuberculosis. The tests may be of some use in prognosis but they do not seem likely to replace good clinical judgment, or even to be of very great assistance to it.

It would seem wise, instead of multiplying these procedures indefinitely, to concentrate on finding out what they depend upon. As already stated, the reagents in most of them are known to contain substances which precipitate globulins under suitable conditions of concentration influenced perhaps by the state of acid base equilibrium. Of three methods to which this statement does not obviously apply, Bonacorsi's depends upon a cholesterolized alcoholic extract of tubercle bacilli, diluted with physiologic salt solution. It is said to give a considerable percentage of agreement with the Sachs-Georgi reaction, which in turn appears to be a nonspecific test for globulin lability. The tubercle bacillus extract is not an essential part of Bonacorsi's solution, according to Kovats.

In 1921 Fornet^{32 34} reported an agglutination test performed with tubercle bacilli defatted with ether vapor at 40°. The technique of the test is not given in full. The "Fornet" diagnostic preparation apparently sold as an agglutination test is said by Bignami to be "a 0.6 per cent carbolic solution of sodium phosphate" containing some more or less acid fast tubercle bacilli, and these are said to be already partially agglutinated.³² Bignami attributes positive results to precipitation of globulin in excess by the acidity of the solution but the carbolic acid alone is sufficient. Larson and Montank

continuing the observations of Stoerck and Porter, have shown that various phenols, cresols, and other chemicals give typical ring tests

"Tubercumet," according to Boissevain and Ryder of the Colorado Foundation, contains a considerable amount of phenol and only a minute amount of nitrogen, yet it gives heavy rings in many cases. The test is probably in essence another phenol test for globulin, certainly in the presence of phenol any other factor cannot be evaluated. Henry and Hatch have published an indefinite account of the preparation of a fractional extract of tubercle bacilli in the manufacture of Tubercumet, but they do not give the chemical composition of the solution in which this extract is dissolved.

The status of these tests seems similar to that of the red cell sedimentation test, which is not an immunologic phenomenon in the limited sense, but depends on quantitative changes in a physicochemical system, apparently involving a relative increase of globulin with reduction of the surface tension of the plasma. When a number of precipitation tests are done on the same bloods they do not agree in all cases with one another, nor with the sedimentation test, but all tend to be positive in the same class of conditions. Schanin and Chrennikow²⁶ in a study of seventy cases of extrapulmonary tuberculosis, found a distinct correlation between globulin increase, reduced surface tension, reduced electric conductivity and dissociation, and increased sedimentation rate, these in turn were correlated with a rise in the isoelectric point and precipitation within wider range of P_H . That is, when 0.01 N lactic acid is added to the serum, precipitation begins nearer neutrality and continues to a higher acidity than is the case with normal sera.

Lehmann-Facijs,²⁷ titrating syphilitic sera with 1:1000 lactic acid, found that in Kahn-positive sera precipitation began with less acid than in normals but the range was not increased, while Sachs-Georgi-positive sera precipitated with less acid than normals and also continued to precipitate to a higher acidity, indicating increased total globulin. He considers that both the amount of globulin and the amount of available ionized hydrogen are concerned in the flocculation. It would seem that the alkali reserve and buffer salts must also have a direct effect on these titrations.

Further study is very desirable with a view to comparing a number of the precipitation tests for tuberculosis and correlating them with the actual globulin content, which is known to be commonly increased in tuberculosis, though it may be diminished in cachexia,^{21 26 28} and it is noteworthy that very advanced cases doing badly often give negative precipitation tests. It may be that all the tests in common clinical use depend on globulin increase solely, in which case it should be possible to select one and place it on a more or less quantitative basis. The work of Daranyi and Baum and Schumann shows a tendency in this direction, though the alcohol-salt solution test may not be the best possible.

On the other hand the relation between globulin concentration and precipitation may be more complex. Reports in general certainly indicate that the flocculation tests commonly used in the diagnosis of syphilis have at least relative specificity, as compared with a simple globulin precipitation test like that with ammonium sulphate, with which Mundel got 100 per cent positive reactions on syphilitic children and 98 per cent on tuberculous children.

Apart from the tests known to depend on simple chemical solutions there remain those in which the reaction, involving an extract of tubercle bacilli, may possibly be an antigen antibody one. Some of the reports of tests using bacillary extracts are impossible to interpret because the authors do not explicitly state that the extract was made with simple physiologic salt solution,⁹ while in other instances, such as Hollaender's,¹⁰ it is stated that phenol is used. On the other hand there are the experiments of Porter, already described, and in 1923 Larson, Montank, and Nelson⁸ reported about 200 cases tested with antigen solutions prepared by disrupting tubercle bacilli with liquid carbon dioxide and filtering. The results showed a fairly high percentage of accuracy. Antigen solutions prepared from acid fast actinomyces, however, gave positive results corresponding to the tubercle bacillus antigens, while those prepared from non acid fast actinomyces gave negatives as did both filtrates from both tubercle bacilli and actinomyces. These antigens were dissolved in physiologic salt solution and Dr. Larson¹¹ is quoted as considering that "the results with blown bacilli seemed to be more specific than with the phenol compounds." A series of tests like Porter's comparing Larson's two methods, one containing bacillary extract but no phenol, the other phenol (triacresol) and no tubercle bacillus extract both in physiologic salt solution, would be of more interest than most of the studies on precipitation tests in tuberculosis which have appeared as yet. It would be well also to pursue the idea of Calmette and Massol, and try to determine what changes occur in the antigen solution and also in serum as the result of the formation of a precipitate.

REFERENCES

- ¹V. Daranyi: *Deutsch. med. Wchnschr.* 1922 p. 17 (cit. v. Kovats)
- ²V. Daranyi: *Wien. klin. Wchnschr.* 1922 p. 45 (cit. v. Kovats)
- ³V. Daranyi: *Tr. Nat. Tuberc. Assn.* 1926 (author's abstract)
- ⁴Enderle: *Ibid.*, 1925 p. 75
- ⁵Pinner: *Am. Rev. Tuberc.*, 1924 x No. 4, p. 441
- ⁶Baum: *Ibid.* p. 449
- ⁷Baum and Schumann: *Fortschr. d. Med.*, 1922 (cit. Baum and Pinner)
- ⁸Larson, Montank, and Nelson: *Proc. Soc. Exp. Biol. and Med.* 1923, xx No. 6 p. 350
- ⁹Montank: *Ibid.*, 1924, xxi No. 9 p. 547
- ¹⁰Montank: *Journal Lancet* February 1925 p. 62
- ¹¹Myers: *Am. Rev. Tuberc.*, 1925, xi No. 1 p. 71
- ¹²Stewart and Collins: *Am. Jour. Dis. Child.* September 1926 p. 367
- ¹³Henry and Hatch: *Tr. Nat. Tuberc. Assn.* 1926 p. 333
- ¹⁴Baum and Black: *Beitr. z. Klin. d. Tuberk.*, 1926 lxx 429
- ¹⁵Matefy: *Med. Klin.*, 1923, (cit. v. Lemesic and Kosanovic)
- ¹⁶V. Lemesic and Kosanovic: *Beitr. z. Klin. d. Tuberk.* 1925 lxx 277
- ¹⁷Lange and Heuer: *Deutsch. med. Wchnschr.* 1924, (cit. v. Lemesic and Kosanovic)
- ¹⁸Mündel: *Beitr. z. Klin. d. Tuberk.* 1924 lxx 623
- ¹⁹Bonaccorsi: *Gior. di Clin. Med. Fasc.* 1922 (cit. v. Kovats)
- ²⁰V. Kovats: *Beitr. z. Klin. d. Tuberk.*, 1924 lxx 64
- ²¹Bignami: *Ztschr. f. Tuberk.* 1924 xli 194
- ²²Porter: *Jour. Infect. Dis.* 1910, vii 87
- ²³Stoerck: *Wien. klin. Wchnschr.*, 1909, lx 417 (cit. Porter)
- ²⁴Calmette: *L'Infection bacillaire et la tuberculose* 1920 pp. 306 and 561
- ²⁵Calmette and Massol: *Acad. d. sc.*, July 4 and 25 1910 (cit. Calmette)
- ²⁶Schanin and Chrennikow: *Beitr. z. Klin. d. Tuberk.* 1926 lxx 759
- ²⁷Lehmann-Faure: *Ztschr. f. Immunit.* 1926, xlii 137
- ²⁸Wells, Dewitt and Long: *The Chemistry of Tuberculosis*, Baltimore, 1923, p. 194. With references to Adler, etc.
- ²⁹Smecton: *Am. Rev. Tuberc.*, 1922 vi, 588
- ³⁰Hollaender: *Ztschr. f. Tuberk.*, 1920 xxxii, 257

³¹Larson Personal communication to C T R

³²Hippe Ztschr f Tuberk, 1926, xlv, 143, with references to Sachs, etc

³³Fornet Ann de l'Inst Pasteur, 1921, xxi, 797

³⁴Fornet Deutsch Arch f klin Med, 1921, cxxviii, 229

³⁵Pinell Compt rend Soc de biol, 1926, xcv, 1319 Abst, Jour Am Med Assn, 1926, lxxviii, 765

—G B W (C T R)

The Reactions of the Omentum

THE apparent facility with which the omentum finds its way to an inflamed area on a foreign body in almost any location within the abdominal cavity has led many to believe that the mechanism of this movement must be a purposeful one rather than dependent entirely upon hazard. Some of the terms applied to the omentum give voice to this impression. Thus we read of the abdominal policeman, the friend in need, the great leucocyte.

Some have attributed to it a power of intrinsic movement. They believe that it has special stretching and contractile capacity. Rutherford Morrison states in his *Introduction to Surgery* that the omentum travels around the abdomen with considerable activity and is attracted by some sort of information to neighborhoods in which mischief is brewing. Saint¹ explains the movement as a phenomenon of chemotaxis. Norris,² however, has shown that the omentum contains no muscle fibers and possesses no inherent motile power.

The weight of evidence at present would indicate that the movement of the omentum to an inflamed area is purely passive. Adam,³ Wilkie,⁴ and Durham⁵ believe that the omentum is carried along chiefly by intestinal peristalsis.

Florey and Carleton⁶ present a convincing series of experiments corroborating the theory of passive movement. Mice and decerebrate cats kept immobilized in the dorsal position for periods of from one hour to two days after the beginning of the experiment, were given intraperitoneal injections of carmine pigment. They then found that only the portions of the omentum which dipped down into the side of the abdominal cavity contained carmine granules. The omenta of control animals which were allowed to resume their normal activities after the injections succeeded in collecting nearly all of the material. Similar results followed the insertion of large foreign bodies such as pith and cotton wool into the abdominal cavity.

Experiments with living bacteria on immobilized decerebrate cats, in which abscesses were produced in the intestinal walls and on the liver surface showed that here again no evident attempt was made by the omentum to reach the areas of inflammation. Cotton wool soaked in a culture of *Staphylococcus albus* was placed in the closed end of a glass tube. The open end of the tube was inserted into the abdominal cavity. The omentum made no attempt to reach the infected foreign body through the open tube. Chemotaxis apparently played no part.

From observation through a window inserted in the abdominal wall the authors concluded that the posture was probably the main determining cause of the apparent movement of the omentum to sources of infection. By bending

the cat they observed that a selected portion of the omentum could be made to alter its position by as much as three centimeters. In this way it could be moved into various positions. Diaphragmatic excursion appeared to play little part. Peristalsis moved parts of the omentum and it would be possible to account for its rolling up around foreign bodies by movement imparted by peristalsis.

The omenta of a cat and a rabbit delivered from the abdominal cavity and observed in a glass cell, with blood supply undisturbed made no attempt to envelop a piece of cotton wool in contact with them or a piece of cotton wool soaked in a culture of *Staphylococcus albus* placed two or three millimeters from them. The observation extended over a period of sixteen hours. Negative results were likewise obtained with the excised omentum *in vitro*.

The evidence against intrinsic movement of the omentum appears quite conclusive. And yet it is remarkable with what facility the omentum becomes attached to the site of a local inflammation or to a foreign body. Peristalsis probably plays a part but body movement appears to be the most important factor. It has been remarked that the bent posture of one suffering with severe abdominal pain facilitates the omentum reaching well down into the pelvis. Twisting and squirming movements likewise appear to be not without logical reason.

Once the organ has established contact the pathologic picture is distinct. At the point of contact a fibrin exudate forms and within an hour or so quite firmly anchors the omentum to the point of pathologic change. Soon leucocytes migrate into the fibrin layer and where pigment granules are present they actively engulf them. The fibrin clot later becomes organized by fibroblasts and new vessels grow out into the recently organized tissue. At the end of from three to five days the site has often become highly vascularized. As a last stage collagen fibers are produced by the fibroblasts and the damaged area or foreign body becomes encapsulated. With the disappearance of fibrin and the epithelialization of the marginal contact surfaces the reaction is completed.

The heavy vascularization of omental adhesions is not infrequently of great value. An outstanding example is in the providing of collateral circulation in cirrhosis of the liver.

LePlay⁷ found that even the resected omentum when left in the peritoneal cavity retains the power to react to foreign bodies.

Recently the possibility of a chemotactic factor has been again raised although in a somewhat different form. Sauarelli⁸ has studied a curious phenomenon which he designates as galvanotropism. He like others has observed the rapid engulfing of particles of earwax or charcoal or lycopodium spores by the omentum. He has found that cholera vibrios pass rapidly into the omentum where they accumulate and agglutinate. He, also discards in great part the theory of intestinal movement or intraperitoneal currents and suggests that the contact of the omentum with a foreign body such as bacteria, may be dependent on some physicochemical process, a colloidal reaction between the peritoneal serosa acting as gel and a microbe or powder which, suspended in the fluid is charged electrically. He recalls that bac

terria, colloridal granules, and infusorial substances possess an electric charge varying according to the nature of the material. Indeed a recently suggested classification of bacteria is based on the electrical reactions of the various microorganisms.

Sanarelli observed that if an omentum is removed from a rabbit or guinea pig and placed in distilled water and then is touched with a clean platinum needle, the organ adheres to the platinum with considerable force. Once rolled about the needle it is separated from it with considerable difficulty. He believes that the omentum charged positively is attracted to the platinum which carries a negative charge. The omentum being a colloidal membrane follows the laws of colloids. If a surface colloidal membrane, chemically inert, comes in contact with a mineral salt dissociated in water into its respective ions, the membrane combines with the latter to form a complex colloid. Thus, the colloidal gel becomes chemically active acquiring the qualities and properties of ions.

The surface of the omentum normally carries a positive electric charge. This can be neutralized by prolonged immersion in sodium chloride solution or in dilute hydrochloric acid after which the omentum no longer adheres to a platinum wire. The reaction is, however, reversible and the power of adhesion will return after prolonged immersion in distilled water. Sanarelli observed a similar reaction in a study of lymph glands but none with such tissues as muscle, liver, kidney and spleen.

The phenomenon is of interest. The author's hypothesis may be correct. Its application as an explanation of the clinical processes under consideration will of course require substantiation.

Omental function is not limited to the removal of foreign bodies from the peritoneal cavity or to its protective adhesion to areas of inflammation. The omentum is active in the absorption of fluids from the abdominal cavity. Wilkie⁴ found that salt solution introduced into the abdomen of animals whose omentum was intact was absorbed half again more rapidly than in animals in whom the organ had been removed. The omentum possesses distinct bactericidal properties. It contains large numbers of phagocytes both scattered through it and in nodal accumulations along the blood vessels. Wilkie injected broth cultures and found that the omentum still contained viable organisms after the peritoneal cavity itself had become sterile. Portis⁷ has attempted to show that the omentum assists in antibody production. There is some evidence that this is the case in the rabbit but he concluded that the evidence in the dog and guinea pig was decidedly less convincing.

The omentum aids in the vascularization of tumors and of organs damaged by infarct. Mechanically, it probably serves also a function somewhat analogous to a ball bearing, in that it interposes a lubricated movable system between the intestines and the parietal peritoneum.

It contains a network of freely anastomosing blood vessels without a large capillary bed. Rich¹⁰ has, however, demonstrated the existence of a fairly extensive capillary bed in the omentum of animals whose capillaries have been dilated following histamine shock. The larger blood vessels are surrounded by layers of fatty tissue. The thinner portion consists of cells

enmeshed in a mass of interlacing, connective tissue fibrils and covered by flattened mesothelial cells. In the rabbit we find a special structure, the *taches lacteuses*, aggregations of various types of free cells in close contact with areas of increased vascularity. In the guinea pig the *taches lacteuses* are replaced by glomerulus like bodies with characteristic central capillary networks and peripheral cellular aggregations. In the dog there is neither *tache lacteuse* nor glomerulus like body. The vessels form extensive anastomoses throughout and there are small cellular aggregations of phagocytes along the vessels. Both the *tache lacteuse* of the rabbit and the glomerulus like body of the guinea pig are characterized by their abundant blood supply each being provided with capillary tufts with afferent and efferent vessels. The glomerulus like bodies usually have two afferent terminal arteries with one efferent vein and a rather extensive intervening capillary network.

The various types of cells observed in the omentum have received close study. For many years it was thought that the fibroblasts and the serosal lining cells were interchangeable. It was thought that in the mesothelial cells were shed in acute inflammation their places were taken by fibroblasts which eventually formed new serosal cells. Kivono was perhaps the first to deny the supposition. Cunningham¹¹ has shown apparently conclusively that the fibroblasts and the serosal cells are distinct and always remain so.

A third type of cell found in the omentum is the leucocyte and a fourth the clasmatocyte. This latter resembles the leucocyte in general appearance but possesses dendritic processes which for a time led to the belief that they were derived from the fibroblasts. Maximow¹² however demonstrated that the clasmatocyte is a distinct type of cell. He introduced two sterile cover slips under the skin of a rabbit and determined the speed with which various types of cells passed between the covers. The leucocytes appeared first then the clasmatocytes which wandered in after about nineteen hours. These were followed later by the fibroblasts. Furthermore the clasmatocyte is especially sensitive to certain dyes particularly neutral red. It appears to be a cell of connective tissue origin specifically differentiated to take up and store particulate matter. Apparently it originates primarily from endothelium.

Portis⁹ injected India ink into the peritoneal cavities of rabbits and removed the omentum after twenty four hours. On microscopic study the ink was found contained chiefly within large phagocytic cells most numerous in the *taches lacteuses*. Similar results were obtained with a 5 per cent suspension of carmine. The results were the same after the injection of chicken red blood corpuscles whose nuclei could be clearly identified. By simultaneous intravital staining with trypan blue the author demonstrated that the leucocytes and particularly the clasmatocytes were the active cells phagocytizing the foreign particulate matter.

Portis found that twelve hours after the injection of 10 cc of a mixture containing 5 cc of defibrinated chicken blood and 5 cc of a 25 per cent acacia suspension in a rabbit the fluid was practically all absorbed but that many smaller and larger clumps were adherent to the omentum. Stained preparations showed many chicken red cells adherent to the external surface of the

omentum and a few intact erythrocytes contained within the vitally stained phagocytic cells in the *taches laiteuses*

After twenty-four hours these latter cells were loaded with the nuclei from the chicken corpuscles while the cell bodies of the corpuscles could no longer be recognized. After forty-eight hours there was considerable fragmentation of these nuclei so that they were almost unrecognizable. Finally, at the end of ninety-six hours only a considerable amount of granular material could be found in the cytoplasm of the cells.

It is of interest that in rabbits who had twelve days previously received 5 c.c. of defibrinated chicken blood, this whole process was speeded up so that at the end of six hours the clasmatoocytes were found loaded with chicken corpuscle nuclei, and the granules had nearly disappeared by the end of twenty-four hours. These previously immunized rabbits were found to take the trypan blue vital stains more extensively and there appeared to be more clasmatoocytes present than in the controls.

This increased response after previous sensitization was by no means as pronounced in guinea pigs as in rabbits. The results correspond with the lessened ability of the guinea pig to produce antibodies.

Vaughan¹³ has shown that the local inflammatory reaction to bacteria, once they have penetrated the omental tissues, does not differ in any important respect from similar reactions in other tissues of the body. Bacteria may penetrate the peritoneum elsewhere than in the omentum, but this appears unusual. Probably the greater motility of this organ and the flexibility of its covering are factors.

The omentum is an abdominal organ of greatest importance, and yet surprisingly little reference is made to it in the standard works on pathology. Indeed the surgeon on the whole may be said to know more of the pathology of this tissue than does the pathologist. The possibilities for productive studies on the omentum should be particularly alluring to the immunologist.

REFERENCES

- ¹Saint Clin Jour 1915, xlv, 172
- ²Norris U of Penn Med Bull, 1908, vi, 119
- ³Adam Lancet, 1913, i, 663
- ⁴Wilkie Brit Med Jour, 1911, ii, 1103
- ⁵Durham Jour Path and Bacteriol, 1897, iv, 383
- ⁶Florey and Carleton Jour Path and Bacteriol, 1926, xxxv, 197
- ⁷LePlay Compt rend Soc de biol, 1911, lxxv, 484
- ⁸Sanarelli Ann Inst Pasteur, 1926, xl, 161
- ⁹Portis Jour Infect Dis, 1924, xxxiv, 159
- ¹⁰Rich Jour Exper Med, 1921, xxxiii, 287
- ¹¹Cunningham Bull Johns Hopkins Hosp, 1922, xxxiii, 257
- ¹²Maximow Beitr d Path Anat u d allg Path, 1901, iv, 1
- ¹³Vaughan, W T To be published.

—W T V

Cults and Their Relation to Medical Practice

AS LONG as credulity, ignorance and unscrupulousness are human attributes cults and irregular, not to say weird and absurd methods for the treatment of disease will arise and flourish for a time to give their place to another offspring of absurdity and sesquipedalianism

Were it not for the regrettable and often disastrous aftermath so frequently visited upon the victims of charlatanry there would be little reason to dignify these fads and fancies by comment or discussion

Inasmuch, however, as the compelling interest of the medical profession now lies as much in the evolution of means for the prevention as well as the treatment of disease and as the achievement of both is closely related to the measure of their general and public understanding, some consideration of cults in their relation to medical practice is necessary in any consideration of the public health

Practically all irregular methods of treating disease are founded and thrive upon the fact that the average man or woman has only the haziest idea of the functions of the human machine and the mechanism whereby they are performed. It is true that there are courses so called, in physiology in the schools, and it is equally true that for every ounce of information in the possession of the average adult, there is a pound of misinformation to more than counter balance

It matters not how skilled the individual in the arts or sciences, how learned in the higher mathematics or in the mazes of law or philosophy, let him be sick and the most amazing abysses of misconception are often brought to light

Physicians are often amused and sometimes astonished to find a patient otherwise well informed and intelligent who gives a history of treatment by various irregular and sometimes absurd methods for the subjects of the oscillo elast, even as the by gone champions of the Perkins tractors are by no means invariably of the "ignorant classes". One often hears astonishment at the credulity so displayed but seldom is there any evidence of appreciation of the relation of the doctor to, or his degree of responsibility for the situation as it exists.

The average human being pays very little attention to his bodily mechanism as long as it functions without undue friction and the average individual is seldom "sick" until his disabilities and functional disturbances have reached a stage where they interfere with his normal and accustomed habits. Then he seeks the doctor, and the same man who hesitates twice and thinks thrice before selecting his broker, banker, lawyer or even his tailor very often steps blithely through the first door labeled "Doctor"

This is not as astonishing as at first glance it appears. The client first visiting a lawyer does not expect an immediate decision. Authorities must

Errata

In the May issue, article by McGuigan, "The Pharmacology of Iron and Aluminum in Relation to Therapeutic Uses," the last sentence in line 20, p 792, should read

According to this aluminum is ten times less toxic than ferrous sulphate, which is much less toxic than ferric salts

In the June, 1927, issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, in the article by Dr S L Leiboff entitled, "A Note on the Measurement of Blood for Chemical Examination," the last two lines on page 912 are transposed

In the June issue of the Journal, in the article by R B Barton, "The Endothelioid Cell in Acute Leucemia," the following corrections are noted in the text

On page 856, line 31—23.5 per cent should be 25.5 per cent

In line 32—46 per cent should be 49 per cent

In the Table on page 857—in the first blood count

Plasma cells should be 0.4% instead of 0.2%, and the Neutrophilic myelocytes should be 8.8% instead of 4.8%

In the second blood count

Myeloblasts should be 25.5% instead of 45.5%

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN M.D. Editor in Chief
Medical Arts Building
Richmond Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON M.D.
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD M.B.
University of Toronto Toronto

Clinical Pathology

W. C. MACCARTY M.D.
Mayo Clinic Rochester Minn.

Biochemistry

VICTOR C. MYERS Ph.D.
State University of Iowa Iowa City

Immunology

JOHN A. KOLMER M.D.
University of Pennsylvania
Philadelphia

Bacteriology

A. PARKER HITCHENS M.D.
Bureau of Science Manila, P. I.

Pathology

PAUL G. WOOLLEY M.D.
Los Angeles

Tuberculosis

GERALD B. WEBB M.D.
Cragmor Sanatorium Colorado Springs

HAROLD D. CAYLOR M.D.
Mayo Clinic Rochester Minn.

Experimental Medicine

RUSSELL L. HADEN M.D.
University of Kansas Rosedale

ROBERT A. KILDUFFE M.D.
Atlantic City N. J.

Surgery

DEAN LEWIS M.D.
Johns Hopkins University Baltimore

Internal Medicine

GEORGE R. HERRMANN M.D.
Tulane University New Orleans

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY 3523 25th PINE BLVD. ST. LOUIS

Entered at the Post Office at St. Louis Mo. as Second Class Matter

The New Fifth Revised Edition of a Popular Book

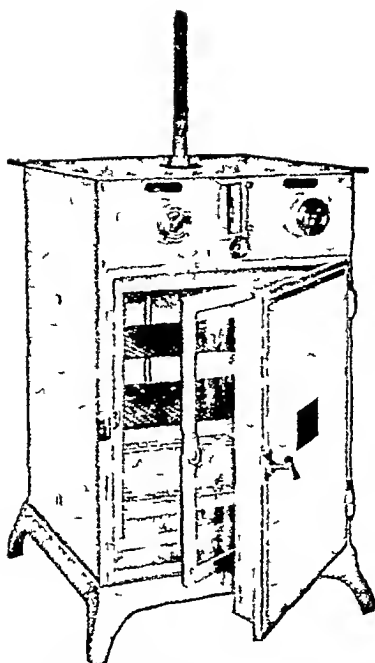
Physiology and Biochemistry in Modern Medicine

Over 1100 pages, 6 1/2 x 9 1/2 with 500 illustrations including half tones and line drawings and 9 color plates Price silk cloth binding, \$11.00

BY J. J. R. MACLEOD M.B. LL.D. D.Sc. F.R.S.

Professor of Physiology in the University of Toronto Formerly Professor of Physiology in the Western Reserve University

Assisted by Roy G. Pearce A. C. Redfield N. B. Taylor J. M. D. Olmsted and by others.



Freas Incubator No 26

Your Incubator Must be Accurate

Just as with your laboratory worker your incubator must be capable of performing the assigned tasks accurately

Accuracy in an incubator is its ability to maintain the temperature constant continuously

This is why the Freas Incubators are used in the leading laboratories

The Thermo Electric Instrument Co.
1215 South Grove Street, Irvington, N. J.

FREAS
ELECTRIC INCUBATORS

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO., AUGUST 1927

No 11

CLINICAL AND EXPERIMENTAL

THE AMEBA COUNCILMANIA LAFLEURI, ITS APPEARANCE AND CLINICAL IMPORTANCE*



By RAWSON J. PICKARD, M D., SAN DIEGO, CALIFORNIA

IT IS difficult to distinguish the different species of amebae parasitic in man. They are small, they vary in number from day to day, protozoal infestation is often multiple, and degenerated forms are common. Diagnosis from the nuclear structure is complicated by the frequency of mitotic figures. The reaction to staining changes with the fecal reaction. Repeated examination of stained preparations is often necessary for diagnosis, and this means the study of the stained cysts, because of their more constant morphology and greater specific difference. The method of choice is the examination of both the fresh motile amebae and that of the motile and encysted forms stained in iron hematoxylin, as between one of the two examinations there can be no question that an accurate diagnosis must be based on study of the stained cysts, and that laboratories not equipped to do this should not attempt the diagnosis of the fecal protozoa.

But the difficulties are not all self created by a desire for rapid diagnosis nor due to lack of experience of the microscopist nor to the difficulties of the technique of wet fixed smears. There is the difficulty of attempting to follow the texts of the authorities and this because the description of the two well known amebae is confused by including the description of a third less known species which latter once recognized permits definition in the description of all three and the ready separation of them from each other. Wenyon¹ says that an ameba 15 to 20 mu or larger very active containing red blood cells and an indistinct nucleus is certainly *Entamoeba histolytica* and 'the general rule holds for practical purposes that an ameba with included red cells is *E. histolytica*'.

tolytica'' A slow-moving ameba with granular pseudopodia, food vacuoles containing yeasts and bacteria but never blood cells, with an eight-nucleate cyst is *E. coli*

What then of the nearly 8 per cent of patients having intestinal symptomatology (Kofoid's figures)² in whom there is present an ameba with, as constant characteristics, the size, great activity, clear pseudopodia, and occasionally ingested red cells of *E. histolytica*, and yet which, like *E. coli* has an eight nucleate cyst and, in the motile amebae, visible nuclei and food vacuoles which contain yeasts and bacteria as well as erythrocytes⁹ Do these make up the indeterminate infection noted by Wenyon (1 per cent to 17 per cent) in his stool surveys as "*Entamoeba* sp (?)'' Certainly this ameba is neither the *E. histolytica* (dysenteriae) nor *E. coli* as defined by Brumpt³ or Dobell and O'Connor⁴

Before Kofoid and Swezy⁵ described *Councilmania laffleur* this ameba was mistaken in the motile stage for *E. histolytica* and in the encysted for *E. coli* Previous to the period of careful observation and interest in the intestinal protozoa which resulted from the wartime surveys showing the world wide frequency of these infections in all classes of the population and their causation of low-grade illnesses, only these two amebae were generally recognized in the feces Cysts were seldom sought or recognized in the clinical laboratories and stained preparations rarely attempted At present with attention centered on the fecal protozoa as one of the sources of the focal infections more exact work is requisite and is more often done

Councilmania has been included with the other amebae, as atypical, figures of this ameba appearing in the texts under other names (eg, Brumpt, p 97, Fig 32, F, H) For the clinician *Councilmania laffleur* is a new distinct species, as well defined as either of the others, once *Councilmania* is separated from them Studying the abnormal shapes of the cysts, finding many of them to all appearance budding in the stools, staining the nuclei and finding them to be of a different type from those of the other amebae, one might concede its status as a new genus, as stated by Kofoid, as readily as one concedes a genus for *Endolimax nana*, the least one could do would be to classify it as a new species of the *Entamebae*, differing from the other two as much as they differ from each other One cannot agree with the texts that *Councilmania* is a "synonym of *E. coli*" To this confusion of description in which to the morphologic forms of the two *Entamebae* there are added as exceptional, the forms that are constant in the third species (*Councilmania*), which once separated furnish three clear pictures, some texts add an additional obscurity by purposely passing over details in a work where attention to detail is every thing What criticism is there for the remark that attention to details of nuclear structure "may assist" in a diagnosis? If Kofoid is in error in insisting on details that it is impossible for the less expert clinician to demonstrate, his is at least the error of setting a high standard

Councilmania laffleur was described by Kofoid and Swezy in 1921 and the authenticity of the species, as evidenced by the constancy of the group or characteristics distinguishing it, should be apparent to any microscopist Yet this ameba is not yet generally recognized Wenyon heads his notes on it "an

aberrant form of *E. coli*, ' and thinks it would be quite impossible in ordinary work to distinguish it either vegetative or encysted from *E. coli*. There is only one reply, and that is that ordinary work is not at all acceptable for stool examinations in this country today. It will not separate the small races of *E. histolytica* from *E. nana* for instance. At the same time it is true that *Councilmania* much resembles the two *Eutamebae* nor do they so greatly differ from each other that one can determine the species of each individual seen on a slide. The chief difference from *E. coli* in the motile amebae is the active movement clear pseudopodia and the occasional ingestion of blood cells from *E. histolytica* the large vacuoles containing yeasts as well as blood and the visible nucleus. The cysts differ from those of *E. coli* in the larger karyosome consisting of several granules and the numerous irregularly shaped cysts ellipsoidal rather than spherical, and the prebudding and budding forms a character not found in other cysts. Small free amebae are not uncommonly found near the cysts of *Councilmania* and Kotford has observed the buds emerge on the fresh slide as well as seen them in feces fixed in paraffin where the question of pressure and rupture was absent. Except for an observation of Wenson who saw protrusions from cysts of *E. histolytica* () possibly representing the escape of small amebae from the cyst ' no one has seen the budding of other eutamebae in the intestine, although they must bud in a new host but under conditions as yet not reproduced. The cysts of irregular form and in which budding was seen, were so carefully described by Mathis and Merceur¹² that we can recognize them as *Councilmania* although they wrongly were considered to be schizogonic forms of *E. coli*, an interpretation due to the fixation in sublimate which does not reveal the cyst wall in *Councilmania* except in those cysts evidently destined for another host in which the wall is thick and no tendency to bud is seen. Fixed in Boum's solution following Langdon's technique the wall is always visible due to the retraction of the ectoplasm. It is easy to convince one's self as to the meaning of the budding cysts by search over a stained slide. Not being a protozoologist I cannot have an opinion on Wenson's hint that *Councilmania* does not present sufficient difference for it to be placed in a new genus. True, the cysts of *E. histolytica* and *E. coli* never bud within the bowel but these cysts must bud sometime perhaps the site and time of gemination have had too great importance given to them on account of their absolute diagnostic value. There can be no doubt as to the specific existence of this ameba. Hall and Reed¹³ for instance had no difficulty in separating the cases in which *E. coli* of the *Councilmania* type was harbored in their study of the pathogenicity of these two amebae.

We must not bind ourselves to facts so readily observed because of the delayed acceptance of this ameba by the writers of the textbooks, for the facts resolve a not infrequent perplexity into clarity once they are aligned as seen and not forced into the two old cadres.

We infer from the presence in monkeys of species with identical appearance that *E. histolytica* and *E. coli* have been long present in man and the primates. *Councilmania* if the presence of species of the genus is a proof may have come to man from the rodents as Kessel¹⁴ suggests or gone to them from man whom they have long molested. One might wonder whether this ameba,

hard to distinguish in the active state from the ameba of dysentery, and in the cyst from *E. coli*, might not be a more primitive species linking the two, showing the history of *E. histolytica* evolving into a tissue parasite and the development of the colon ameba as a lumen parasite, whether clear ectoplasm and active pseudopodia are structural modifications of an ameba that feeds on the tissues of its host, perhaps forming a capsule that protects the endoplasm and nucleus from the destructive effects of the histolytic enzyme.

Kofoid found *Councilman* as an infection in 78 per cent of 4763 persons referred to him for examination, most of them patients under a physician's care, there were 38 per cent more with *E. coli*, a total incidence of 116 per cent. The patients referred to my laboratory for fecal examination have nearly all been sent on account of symptoms of chronic amebiasis. Chronic infection with intestinal protozoa would more accurately describe their semeiology. Among the last 43 patients there were 21 with negative feces, with a total of 143 stools examined, and in the 22 patients who had intestinal protozoa there were 30 infections, 10 with *Chilomastix*, 3 with *Trichomonas*, 3 *Giardia*, 1 each of *E. coli*, *Dientameba fragilis*, and a small undiagnosed ameba, probably *Endolimax nana*, 8 with *E. histolytica* of whom 3 had dysentery. Of these two had never lived where dysentery is endemic. One was an eight-year old native of San Diego infected with the small race (stained cysts 4 μ to 5 \times 6 μ , vegetative 8 μ in diameter), the other, aged 45, had lived in the northern midwest and in California.

There were three infections with ameba of the species described by Kofoid as *Councilman* *lafeuri*. Slides from each were sent to Dr. Kofoid who confirmed the diagnosis.

CASE 1—Mrs. P, about fifty years of age, had lived in Colorado before coming to California four years ago, partly for her health, her complaint being weakness, "spells of faintness," and constipation. The last had become obstinate two years previously when an operation was advised at which adhesions were found obstructing the ileum. After a few months the fainting spells recurred with loss of strength and constipation. At this time the feces was examined, numerous *Councilman* were found, both motile amebae containing blood cells, cysts and budding cysts, along with *dientameba* and another (?) small ameba. No other cysts were found. Treatment with stovarsol (not well borne), alternating with emetine, cleared the protozoa so none could be found in a series of stools four months later. There has been practically no clinical change, but the time elapsed is too short to judge.

CASE 2—Mrs. B, in her forties, had always lived in the northern midwest and California. For four years she suffered from a fatigability which kept her in bed much of the day, with pains in the sacrolumbar region, which were diagnosed "toxic" after many thorough examinations. There were found in her stool *Chilomastix*, a few small amebae not positively identified (*E. nana*?), and numerous *Councilman*, motile, encysted and budding. Six weeks after her last treatment with stovarsol in a series of ten stools in nine days, several following salts, it was impossible to find any protozoa. Her condition was definitely improved, and now three months later, her stools are still negative. She feels well and considers herself completely cured.

CASE 3—Miss S, aged forty years, had a secondary anemia (70% Newcomer) that persistently recurred after treatment. In the search for a cause, after several years, the stool was finally examined with the finding of *Trichomonas*, *Chilomastix*, a few small (0.5μ) *E. histolytica* but no cysts, and numerous *Councilman lafeuri*. The *Councilman* had a nucleus that was markedly visible, as noted below. There were numerous cysts, budding.

cysts, a few small escaping amebae 1 to 5 mm in diameter. A month after treatment which had been followed by great improvement he noticed beginning loss of strength and re-examination showed numerous *Councilmaniana* alone. With return to tar-sol treatment she is again clinically well and active in her profession.

CHARACTERISTICS

Fresh—Large numbers of actively motile amebae were present varying in size from 12×15 mu to 55×20 , average about 20×30 , with perfectly clear hyaline ectoplasm from which the pseudopodia were formed. In two cases the endoplasm was vacuolated, somewhat granular, greyish, but less dense than *E. histolytica*, as the nuclei were visible. In Case 3 the endoplasm was but slightly denser than the ectoplasm and in the fresh feces the nucleus was conspicuous among the food vacuoles as a circle (optical section) of bright refractile granules. After the first course of treatment in this case the body of

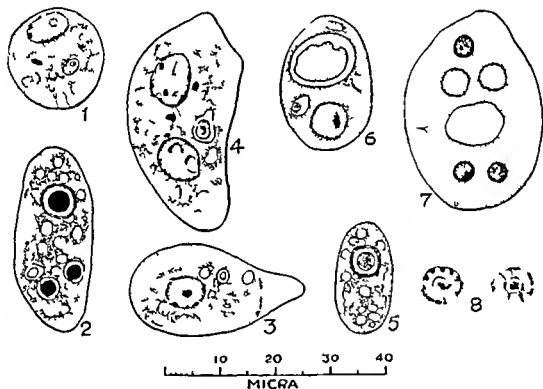


Fig. 1 to 6—*Councilmaniana laffleuri* vegetative. Fig. 4 has two nuclei. Fig. 7 contains two red blood cells.

Fig. 7—Binucleate *E. histolytica* containing three blood cells to show difference of ectoplasm in stained specimen.

Fig. 8—Nuclei of *C. laffleuri* vegetative.

the ameba became still clearer and less dense so that the ameba was hard to distinguish under the lower magnifications and except with dim light. Food vacuoles were conspicuous in all cases, containing yeasts, bacteria, and in 15 to 30 per cent blood cells. Motion was active, the pseudopodia being suddenly formed like those of the dysenteric ameba. The nucleus changed shape during motion. The cysts 15 to 20 mu in diameter were thick-walled. The eight nuclei were readily seen in iodine-eosin stain. Their shape was irregular. Budding and pyriform cysts were as common as spheroids.

Stained—Motile forms. In preparations wet fixed in either hot or cold alcoholic Bouin's solution and stained in iron hematoxylin the vegetative amebae were usually caught in movement and fixed as round-ended cylinders, or with one end stretched out in a long neck. The reaction to stain varied on

the same slide. The endoplasm of some amebae took so deep a stain that the nucleus was obscured and with the clearer vacuoles the ameba looked like a purplish sponge. The ectoplasm did not often show the distinct demarcation one might expect from the appearance in life, when stained it appears not like the sharp bordered fine foam-like ectoplasm of *E. histolytica*, but as irregular lighter vesicular areas on the sides or ends of the ameba, pointed processes of the endoplasm flowing through out to the wall or toward it (Figs 1 to 6). When present in the ameba of dysentery they terminate more sharply (Fig 7). In about 25 per cent when stained the ectoplasm was still distinct (Figs 1, 3). Blood cells (Fig 2) were present in about one-third (of 96 amebae), yeast, bacteria and larger unrecognizable fragments (Fig 6) were in the vacuoles. The stained nuclei varied greatly in the amount of chromatin on the nuclear membrane. In about half (motile) the karyosome was large, over 1 μ . A clear area, the halo, was exceptional about the karyosome. The chrom threads were frequently radial when the karyosome was small. (Fig 8, isolated nuclei)

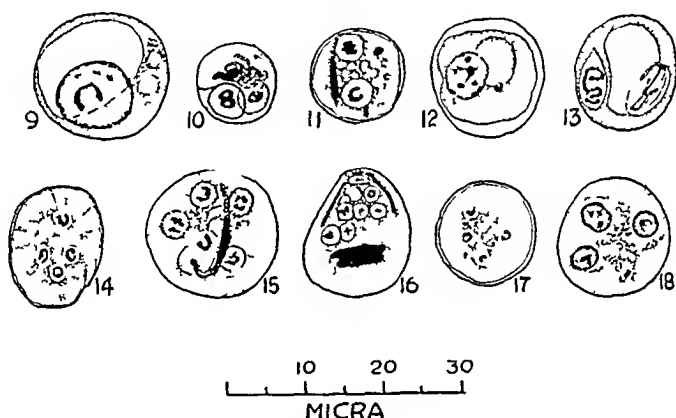


Fig 9—Mononucleate cyst. C. histolytica large glycogen vacuole.

Fig 10—Binucleate cyst. Glycogen vacuole.

Fig 11—With chromatoids.

Figs 12 and 13—Nuclei in mitosis.

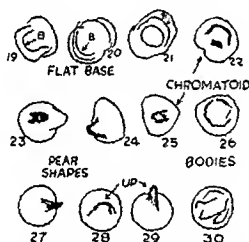
Figs 14 to 17—Eight nucleate cysts. Figs 15 and 16 contain chromatoids.

Fig 18—Four-nucleate cyst in mitosis.

Stained Cysts—The cysts stained irregularly, frequently taking a deep "chromatoidal" stain, like many of the active amebae. These when de-stained lost the stain from the nucleus. Cysts averaged about 17 μ in diameter. Some were pyriform (Figs 23, 24, 27, 28, 29), many cysts had irregular folds (alcoholic Bouin fixation) and rested on the slide upon a flatter area, as seen by focussing (Figs 19, 20, 21), or with a neck or bud pointing up to one side (Figs 23, 24, 28, 29) forming a figure not readily drawn as seen, but obviously not the product of "pressure of the cover glass," "rupture," etc. I found a few cysts that might have been "ruptured." I so thought because the herniated cytoplasm (Fig 32) contained neither chromatoids nor a nucleus, and took a deep counterstain of eosin, which was not true of the gametocytes. Whatever the cause of these ruptures, and there is no evidence that hot fixation ruptures, they took place through a point in the cyst wall that it was not able to suppose was prepared to give exit to a bud. I found nothing

that could be considered the crushing of a cyst by the cover glass, an experiment I have not succeeded in performing. Often buds were found in the thick areas where focussing showed the cover well above the cyst resting on debris. Buds were often found on the upper side of a cyst (Figs 31, 35, 36), and the pear shaped cysts usually were fixed on the slide with the neck up, a position naturally taken by an irregular body with a large base and impossible under pressure. The gemmation cannot be an artefact.

Cysts fixed in hot Schaudinn's solution are more regularly spheroidal or ellipsoidal, are farther shrunken from the fecal debris, and present a more evenly distributed cytoplasm (Figs 15, 17) than those fixed in alcoholic Boun's solution, either hot or cold. In this fixative the cyst wall leaves a narrower clear area between it and the bacteria round about (Fig 42, photograph) than with Schaudinn's. With Boun's often a denser cytoplasmic mass, with the nuclei, is drawn away from the wall, leaving a space perhaps 2.5 μ wide crossed by fine protoplasmic threads giving the area the light, vacuolated appearance of the ectoplasm in the motile Councilmania (Figs 14, 31, 33, 34, 35, 37, 42). But this method of fixation brings out another interesting feature



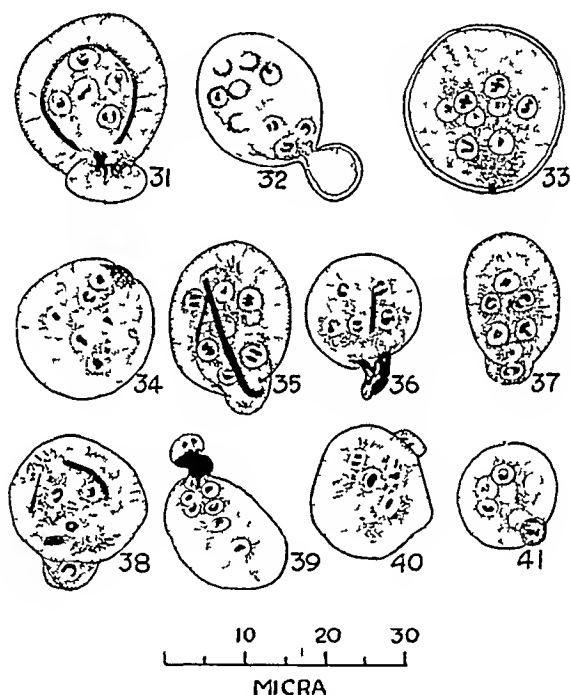
Figs 19 to 30—(Not drawn to scale. Irregular contour of cysts at different levels of focussing are shown in Figs 19 to 21. Figs 22 to 30 show chromatoids of different shapes. Figs 23, 24 and 25 show pear shaped cysts. In Fig 24 and 25 with chromatoids in neck of pear. Figs 27 and 28 have pointed chromatoidal extrusions.

The nuclei and surrounding denser cytoplasm are not always suspended in the center of the cyst. The mass attaches to a point on the cyst wall where may be seen an irregularity, either a dark line through the wall, a "pore" (Fig 33), a depressed area (Fig 34) apparently a break or thinning of the wall, a bulging of the cyst (Fig 16) or the formation of a pyriform neck, or actual gemmation (Figs 31, 35 to 41). The tendency to bud is thus seen in cysts not yet budding and the attachment of the cytoplasm to a part of the wall often morphologically marked shows the frequency and the natural occurrence of gemmation in this species of ameba.

I found budding present in 20 to 40 per cent of the cysts, in about 3 per cent there was a small ameba with one nucleus separate beside the mother cyst with 7 or 6 nuclei remaining (Figs 41 and 42 photograph). Budding cysts often show a massing of nuclei near the bud, chromatoid material was often present in the extrusion ahead of the nuclei (Figs 16, 24, 27, 28, 29, 31, 34, 35, 36). Sometimes the budding process is intensely stained, the border fading toward the cytoplasm (Figs 29, 39). The chromatoid matter may be in sharp

fragments like crystals, or in threads (Fig 30) In a few cysts I have found the chromatoidal staining ridge as described by Kofoid In such a cyst if there was a bud it was situated on the ridge, which lies on the denser cytoplasm within the cyst wall (Figs 15, 16, 31)

The frequency of chromatoid bodies or chromatoidal staining cytoplasm in the buds in the eight-nucleate cysts, and the comparative infrequency of chromatoidals in cysts in which there was an extrusion of cytoplasm containing a nucleus, a new forming ameba, raised a question as to the purpose of the extrusion of the chromatoidal material and the sequence of these events It might be that the material staining like that of the chromatin of the nuclei is condensed from the cytoplasm previous to and as a part of the process of gemmation, and that the cyst which is about to break up into small amebae first con-



Figs 31 to 40—Eight-nucleate except Fig 33 (All nuclei not in focus) Fig. 31 bud with chromatoidal matter chromatoidal ridge encircling cytoplasm Fig 32 bud without nucleus cyst with nine degenerate nuclei Fig 33 cyst with pore Fig 36 chromatoid extrusion in bud Fig 39 same with nucleus in addition

Fig 41—Seven-nucleate Small free ameba beside cyst

centrates and extrudes the chromatoid material Perhaps this is the nuclear food supply It is then followed by the nuclei which must leave the cyst to survive, breaking through the cyst wall in gemmation, each surrounded by a small mass of the "hungry" cytoplasm, and forming small active amebae The formation of the chromatoidal bodies appears thus to be a stimulation to gemmation

The nuclei in the ripe cysts, about 25 mu in diameter, number eight, and have seldom any chromatin on the membrane The nuclei appear as lighter areas in the cytoplasm, sharply defined, with the karyosome as a group or chromatium granules in a mass, circle, V-shaped, or irregular arrangement

Mononucleate cysts (2 per cent, Fig 9) and binucleate cysts (5 per cent, Figs 10 to 13) usually have a large glycogen vacuole, resembling those of *E. coli*. Wenyon suggests that the cysts with glycogen are terminal, never forming 8 nuclei, a dividing nucleus like that in Fig 13 would seem to be growing. Fig 18 is a four nucleate cyst (3 shown) in mitosis. About 2 per cent had 4 nuclei. Those with 5, 6, 7 have budded. The cyst shown in Fig 32 had nine.

Further details can be found in the papers by Kofoid and his associates, and by following their technique pictures similar to their figures can be seen.⁸ I have given above such details as seemed different with my preparations. I feel that the technique of wet fixation in alcoholic Bouin's solution is much simpler than Schaudinn's and gives as good detail although different in some particulars. This method appears more practical for the average laboratory where economy of time and space are essential, and where stool work is only one of a large number of techniques to be followed each day. With this method it is possible, in the press of work to leave a process temporarily incomplete. Theoretically as Langeron has pointed out the alcoholic piciformol with acetic acid is an ideal fixation.¹⁰

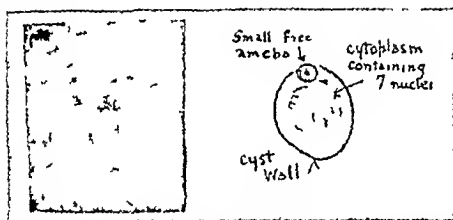


Fig. 4.—Photograph of seven nucleate cyst with small free amoeba to demonstrate.

All figures except Fig. 15 are from preparations wet fixed in alcoholic Bouin's solution and stained from hematoxylin.

I fix slides in alcoholic Bouin's solution cold, for an hour or hot at about 60° C, for five minutes, then follow with 95 per cent alcohol for hardening, following the technique for iron hematoxylin given by Langeron in his *Precis de microscopie*. For cold fixation the flat glass 'Laverin' boxes are best. For the rest of the procedure Coplin or Borrel jars will serve. Half as large a set up is needed for Bouin fixation. One may clear from the 95 per cent alcohol to balsam with creosote where absolute alcohol is difficult to obtain. I seldom get a good stain when in haste.

The amoeba *Councilman* seems to be pathogenic in the manner that the small races of *E. histolytica* are pathogenic, causing low grade neurotoxic disorders and constipation. The cases reported by Kofoid and Swezy were under the care of physicians for symptoms referable to the intestinal tract, 'bowel conscious' as Boyers puts it¹¹ or were subnormal physically. The three reported here were all invalids. Hall and Reed report 11 cases with *E. coli*, and 16 with *Councilman*, with 'neurasthenia', epigastric discomfort, flatulence, constipation, neuralgic pains. On treatment and eradication of the amoebic infection

TABLE I
TABLE OF DIFFERENTIAL CHARACTERISTICS*
MOTILE AMEBAE, UNSTAINED

INDIVIDUALS	C. LAFLEURI NUMEROUS	E. HISTOLYTICA NUMEROUS	E. COLI FEW
Occurrence in "bowel conscious" patients	About 10 per cent (10-15)	About 10 per cent (5)	About 10 per cent (15)
Size	25-35 mu (35-65)	20-30 mu	20-30 mu
Appearance	Greyish, nucleus visible	Bluish, refractile, u invisible	Greyish, n visible
Ectoplasm	Distinct, glassy	Distinct, glassy	No demarcation
Pseudopodia	Clear, broad, single	Clear, several	Granular
Endoplasm	Vacuolated	Granular, uniform	Vacuolated
Food	Bacteria, yeasts, blood cells	Blood cells, feeds by absorption†	Bacteria, yeasts, never blood
Motility	Very active	Very active (small races sluggish)	Sluggish
MOTILE AMEBAE, STAINED			
Ectoplasm	Distinct, or reticulated, with indistinct minute border	Clear, distinct	No demarcation
Nuclear membrane	Thick, chromatin granules fine or coarse	Thin, chromatin granules fine	Thick chromatin in coarse granules
Lioun network	Often radial	Radial threads	Irregular, may hold chromatin
Karyosome	Over 1 mu massed, no halo	0.5 mu, rare 1 Small central granule, with clear halo	1 mu Single granule often eccentric
CYSTS STAINED			
Size	16-20 mu	(6) 7-12 (20)	(10) 15-20 (30)
Wall	Thick, cyst hard to stain, 1 mu	Thin, 1/2 mu	Thick
Shape	Irregular, spheroidal, pyriform, depressions	Spheroidal	Spheroidal
Nuclei	1-2 per cent, large glycogen 2-5 per cent large (vacuole) 4-2 per cent 8 common 16 rare	1-30-45 per cent 2-13-30 per cent 4-25-55 per cent	1 rare 2 rare 4 rare 8 common 16 rare
Peripheral chromatin	None, or thin	Thin, even	Large beads, plaques
Karyosome	Massed or dispersed granules	Single central bead	Single eccentric bead
Chromatoid material	Blunt, sharp pointed or threads, or diffuse edge. Contributes to buds. About 30 per cent	Large blunt masses, 50 per cent	Sharp V 10 per cent splinterlike
Glycogen	Large in precystic, 1, 2 n vacuole	Diffuse, not abundant	Largest vacuole in binucleate cysts
Budding	10-20 per cent, small amebae, 3 per cent	Unknown	Unknown
Fresh, unstained	Nuclei visible in iodine only	Nuclei visible in iodine only	Nuclei faintly visible

*Compiled from Brumpt, Dobell and O'Connor, Kofoid and Wenyon.

†The small races of *E. histolytica* often contain bacteria.

two-thirds were definitely improved, 10 per cent greatly improved. The infections are usually of years' duration, and form, as Bowers insists, but a part of many infectious and degenerative disorders. Improvement is all that can be expected, and a year is not too long to wait for it. Like chronic amebiasis

with *E. histolytica* the infection causes symptoms in midlife and beyond. If there is a well defined effect, like the anemia in Case 3, a result can be expected in a short time. If *Councilmania* like *E. histolytica* infects the gall bladder, as seems likely,¹² it may penetrate to tissues still farther from the intestine.

Kessel thinks the two species of ameba in rats and mice classified by him in the same genus and probably not pathogenic, but in his experiments he found evidence of immunity, which certainly does not exist against a wholly innocuous parasite. Then, it is difficult to recognize a low grade illness in a rodent.

In the volume of literature about the parasitic amebae of man a few attempts have been made to show that *E. coli* is sometimes other than a harmless feeder on the bacteria and yeasts in the intestinal lumen. There have been occasional reports that *E. coli* ingested red blood cells and seemed to have assumed an actively pathogenic role, but they were scattered observations, and of course lacked the proofs that are demanded properly of those who wish to show the pathogenicity of a bacterial species. There was however sufficient to arouse suspicion either that *E. coli* could under certain conditions become a tissue feeder, or that there might be a pathogenic species confused with it.

If *Councilmania lafleuri* Kofoid and Swezy is not an acceptable species then *Entameba coli* must be accepted as becoming pathogenic at times under going at the same time structural changes that are pathognomonic of the change and permanent. If the goddess of science has a form of worship it is the religion of doubt. It is therefore to be expected that a new species like all new findings, must wait for recognition. But if a species is a group of similar organisms readily distinguished from other groups *Councilmania lafleuri* is a valid species.

REFERENCES

- ¹Wenyon. Protozoology. Wood and Co. New York.
- ²Kofoid, Swezy and Kessel. On the Distinctions Between *E. Coli* and *Councilmania Lafleuri*. U Calif Pub Zool 1924 xxxi No 1 21-39.
- ³Brumpt. Parasitologie (new edition to appear May 1924) Masson Paris.
- ⁴Dobell and O'Connor. The Intestinal Protozoa of Man. Wood and Co.
- ⁵Kofoid and Swezy. *Councilmania Lafleuri* a Parasitic Ameba of the Human Intestine, U Calif Pub Zool June 23 1921 xx No 7.
- ⁶Hall and Reed. *Entamoeba coli*, a Chemical Study. Jour Am Med Assn June 20, 1925, lxxxiv 1914-15.
- ⁷Kessel. Amebiasis of Culture Rats and Mice. U Calif Pub Zool Feb 26 1924 xx 489-544.
- ⁸Kofoid, Swezy and Kessel. On the Genus *Councilmania*. U Calif Pub Zool Oct 31 1923 xx 431-445.
- ⁹Laugeron. Précis de Microscopie (Theory and Best Practical Methods) Masson Paris. Stool technique only in 'Coprologie microscopique'. Laugeron and Rondero du Noyer. Paper Masson.
- ¹⁰Kofoid. The Occurrence of Cysts of *Councilmania* in the Duodenal Drainage, Proc Soc Exper Biol and Med 1926, xxxii 299-301.
- ¹¹Boyers, Kofoid and Swezy. Chronic Human Amebiasis. Jour Am Med Assn, Nov 7 1926, lxxxv 1441-1447.
- ¹²Mathis and Mercier. La schizogonie chez les entamoebes intestinales. Bul Soc Path Exotique, 1927, v 311.

TRYPTOPHANE REACTIONS IN THE SPINAL FLUID*

BY BURNHAM SARLE WALKER, PH D, AND FRANCIS HARPER SLEEPER, MD

IN AN attempt to apply the Liebermann-Burchard test for cholesterol directly to the spinal fluid, Boltz¹ observed a color reaction in the fluids of paretics and of some cases of other forms of neurosyphilis. No explanation for this color development was at hand, but on account of its almost uniform incidence with fluids of paretics and tabetics, and its equally uniform absence from the fluids of mental patients with other than a syphilitic condition, it was offered tentatively to the profession as a test of apparent diagnostic value in these neurosyphilitic cases.

The technique of the test as published is as follows: to 1 cc of the fluid to be tested is added 0.3 cc of acetic anhydride, drop by drop, with shaking. To this mixture is added in a similar manner, 0.8 cc of concentrated sulphuric acid. After five minutes of standing, the tube is examined against a white background, and the appearance of a blue or lilac color is considered as a positive reaction.

As a result of the failure to obtain cholesterol reactions with other standard tests, Boltz concluded that the chromogen in this test was not cholesterol, but was some other substance which appeared, or increased in amount, in neurosyphilitic conditions.

Grossman² made a clinical study of the test, showing that it coincided with the Wassermann and other tests used in the diagnosis of general paralysis. He also made the interesting observation that a solution of egg white in water would give a "positive" reaction. In spite of this, however, he interpreted the reaction in terms of minute quantities of cholesterol.

Harris³ also studied the test clinically. Out of 92 cases of general paralysis whose fluids he examined by this method, 89 presented a positive reaction. Two out of five cases of other forms of neurosyphilis were positive. Of other types of mental disease 83 cases were investigated, and only one of these showed a positive response. Harris points out that the agreement here is nearly as good as that obtained with the Wassermann. In regard to the explanation of the reaction, he also inclines to the belief that cholesterol is the substance involved.

A hint as to the nature of the reaction is given us by the work of Aiello⁴ and of Brugi,⁵ published in Italy. They investigated the spinal fluid with the purpose of detecting and estimating the quantity of tyrosine and of tryptophane present. For tyrosine they used the familiar Millon reaction, and found that the amount of tyrosine found paralleled the amount of protein. Their method of estimating tryptophane was based upon a modification of Voisinnet's method proposed by Fuith,⁶ and both Aiello and Brugi seem

*Contribution from the Evans Memorial and Worcester State Hospital
Received for publication March 19 1927

to feel that by using this method they are measuring free tryptophane, and not the combined tryptophane in the protein molecule. The experiments are not reported in detail and it is difficult to evaluate their exact import. At any rate, the Fuith Vorsmet reaction for tryptophane was negative in almost all fluids except those of tubercular meningitis.

The observation of Grossman that the Boltz reaction was positive with egg white solution led the present writers to consider that the Boltz reaction might also be an index of excess protein in the spinal fluid. With this end in view, the egg white experiment was repeated using different strengths of commercial egg albumen standardized by means of nitrogen determinations by the Kjeldahl method. It was found that egg albumen solutions containing 30 mg or more protein per 100 cc gave a positive reaction increasing in intensity with increasing amounts of protein. A solution containing 15 mg protein per 100 cc gave no test whatever with the Boltz reagents, whereas solutions intermediate to these values gave a slight color which was difficult to apprehend with certainty.

From these considerations it becomes apparent that we are dealing in the Boltz reaction with a test for protein or for some constituent of protein. Reference to the work of Hopkins and Cole⁷ gives us very definite information in this respect. Aldehydes are present as impurities in all ordinary samples of glacial acetic acid and of acetic anhydride. In the presence of concentrated sulphuric acid the aldehydes react with the tryptophane groups of any protein which contains such groups producing the characteristic lilac or blue color (the Adamkiewicz reaction). Hopkins and Cole found that one of the aldehydes present in glacial acetic acid or acetic anhydride is glyoxylic acid and that solutions of this acid or its salts would give the characteristic reaction.

Hence to verify our conclusion that the Boltz reaction is a test for protein we substituted for the acetic anhydride of the original Boltz procedure, a solution of the magnesium salt of glyoxylic acid prepared according to the method of Benedict⁸ and obtained positive results with pruritic fluids and with egg albumen solutions.

Since the intensity of the color developed in the reaction was found to be proportional roughly to the amount of protein present it was possible to prepare standard solutions the color of which could be used as a basis for classifying the reactions obtained from spinal fluids. Thus the color obtained with a solution of egg albumen containing 30 mg per hundred cc (standardized by Kjeldahl determination of total nitrogen) was designated as +. A solution twice this strength gave a somewhat stronger color, called ++ and a solution containing 120 mg protein per 100 cc was taken as the +++ standard. Since the majority of the fluids studied did not reach this strength and none surpassed it it was found unnecessary to continue the standards beyond this level.

To check the results of the Boltz test against the actual amount of protein in the fluids under investigation the protein in these fluids was determined by a modification of the Folin micro-Kjeldahl method suggested by the work of Lang.⁹ One cc of the fluid was measured into a 50

TABLE I

MACRO KJEHLDAHL MG N PER 100 C C	PROTEIN PRECIPITATION AND DIGESTION	DIRECT DIGESTION
10.4	10.1	10.6
30.2		30.2
20.3		21.4
5.2	5.3	

c c pyrex centrifuge tube, and about 7 c c of water added. To the diluted spinal fluid was then slowly added 1 c c of the sodium tungstate solution and 1 c c of the 2/3 normal sulphuric acid, these solutions being the same as are used for the precipitation of proteins in the Folin-Wu system of blood analysis. The tubes were then allowed to stand, after mixing, for an hour or more, and then centrifuged for five minutes at a moderate speed. The supernatant liquid was poured off the precipitated protein, and the tubes

TABLE II*

PATIENT	PROTEIN	NPN	BOLTZ	WASS	GOLD CURVE	DIAGNOSIS
1	167	14.8	+++	pos	5555543000	General paralysis
2	120	13.2	+++	pos		" "
3	77	19.7	+++	pos	5444330000	" "
4	61	12.8	++	pos	1122332100	G P (malarial treatment)
5	60	12.2	+	pos		General paralysis
6	59	25.1	++	pos	5543000000	" "
7	55	22.8	++	pos	5555543321	G P (cardiorenal complications)
8	47	21.1	+	neg	0012210000	Involuntary melancholia
9	45	17.2	++	neg	3422100000	Cerebrospinal syphilis
10	44	20.3	+	neg	0000110000	Not diagnosed
11	42	9.5	+	neg		Dementia precox
12	42	13.7	+	neg	1110000000	Manic depressive, manic phase
13	41	11.9	±	neg		Senile psychosis
14	38	14.8	+	pos	5543200000	G P (malarial treatment)
15 (Dec 8)	38		+	neg	0000000000	Manic depressive,
15A (Dec 1)	37		+	neg	0033320000	manic type
16	37	13.4	+	neg	1122000000	Undiagnosed psychosis
17	36	13.5	+		0012000000	Unclassified psychosis
18	35		+	neg	0000000000	Dementia precox
19	33	8.8	±	neg	0000000000	" "
20	31	11.9	+	neg	1221000000	D P catatonic
21	29	14.0	+	neg	1112000000	D P paranoid type
22	28	14.8	±	neg	0001100000	Not diagnosed
23	27		+	neg	0000000000	Senile psychosis, paranoid type
24	27	12.9	±	neg	0011000000	Not diagnosed
25	26	20.3	±	neg		Epilepsy, syphilis
26	24	21.5	±	neg		Psychosis, alcoholism
						ch deterioration
27	20	20.9	+	neg	0001110000	Not diagnosed
28	19	13.2	+	neg	0011000000	Not diagnosed

*The figures for protein and for nonprotein nitrogen are in mg per 100 c c spinal fluid.

inverted over a filter paper until dry. The precipitate formed was then digested in situ with 1 c c of Folin's undiluted digestion mixture, exactly as in the determination of the nonprotein nitrogen of the blood except for the stronger acid. After digestion the contents of the tubes were washed into 100 c c volumetric flasks and Nesslerized with 30 c c Nessler's solution, the excess being necessary to neutralize the highly acid digestion mixture. The standards for color comparison were 0.05 mg and 0.10 mg nitrogen in the

form of ammonium sulphate. One cc of the digestion mixture was added to each flask containing standard solution, and 30 cc Nessler's used as in the unknowns.

Simultaneously with the above determination of the protein nitrogen a micro Kjeldahl was run on a 1 cc sample of the spinal fluid to determine the total nitrogen. By difference of these values it was then possible to calculate the nonprotein nitrogen.

The accuracy of the above method of determination of the protein nitrogen was checked by determinations of the protein nitrogen in egg albumen solutions which had been standardized by macro Kjeldahl nitrogen determinations.

Table II gives the results of our determinations on spinal fluids, arranged in order of decreasing amounts of protein found.

From inspection of Table II it becomes evident that there is a definite correlation between the amount of protein present and the intensity of the Boltz reaction. Thus the mean of the protein values of the three +++ cases is 121 mg per 100 cc, which corresponds exactly with the egg albumen standard of 120 mg per 100 cc. The mean of the four ++ cases is 55 mg per 100 cc, again agreeing closely with the standard of 60 mg. Similarly, the mean of the 16 cases reacting + is 36 mg per 100 cc, with 30 mg as the standard.

An exact agreement is hardly to be expected since egg albumen does not exactly agree with serum albumen or serum globulin in tryptophane content, and the protein of the spinal fluid has been known for some time to be a variable mixture of albumen and globulin. Furth⁶ gives figures showing that the tryptophane contents of these substances are not widely divergent, however.

Egg albumen contains	26 per cent tryptophane
Horse serum albumin	12.15 per cent
Horse serum globulin	4.147 per cent

Thus it happens quite fortuitously that egg albumen occupying a median position in regard to tryptophane content between albumin and globulin, makes an ideal standard for the rough determination of mixtures of the two.

The doubtful cases (those indicated by the sign \pm) fall into two categories: some are weak reactions where the color was so faint as to be difficult of apprehension; others are reactions where some interfering substance in the fluid caused the development of a brown color which masked the lilac tint of the reaction. One possible interfering substance would be sugar in excess; it was found that the addition of a few drops of 1 per cent glucose solution to the standard egg albumen solution before performing the test would cause such a brown coloration to appear upon the addition of sulphuric acid.

In no case did we obtain a frankly negative reaction. There was always some change in the color of the fluid upon the addition of the reagents, and in most cases this could be seen as a pink or lilac tint. The only cases where this was not observed were those in which the development of the brown color

took place This is compatible with the knowledge which we have as to the presence of protein in the spinal fluid, some protein being always present, which gives the faint reaction In general paralysis and in other conditions where the protein content of the spinal fluid is increased we find a marked increase in the color development with the Boltz reagents

From these considerations we must conclude that the Boltz reaction, used in conjunction with standards made up from egg albumen solutions of known protein content, is of value in roughly estimating the amount of protein in the spinal fluid, and that it cannot be considered as being strictly specific for general paralysis or even for neurosyphilis, inasmuch as extreme increases in spinal fluid protein occur in other conditions, e g, typhus fever, and certain cases of disseminated sclerosis, to choose from widely separated fields

SUMMARY

1 The Boltz reaction has been shown to be dependent upon the tryptophane present in the protein molecule

2 It has been demonstrated as varying in intensity with the amount of protein present in spinal fluids and in egg albumen solutions of known protein content

3 This relationship has been found not to be strictly quantitative, as a result of varying tryptophane content and of the presence of interfering substances in varying quantity

4 It is suggested that the Boltz reaction may be of considerable value in estimating increases in the protein content of spinal fluid, although it cannot be considered as specific for any single clinical entity

REFERENCES

- ¹Boltz Am Jour Psychiat, 1923, **111**, JOUR LAB CLIN MED, 1926, **vi**, 1187
- ²Grossman Jour Ment Sc, 1925, **lxii**, 439
- ³Harris Brit Med Jour, 1926, No 3395, p 136
- ⁴Aiello Policlinico S P, 1922, **xviii**, 537
- ⁵Brugi Pensiero Med, 1923, **xii**, 583
- ⁶Furth and Nobel Biochem Ztschr, 1920, **cix**, 103, and Lieben, *ibid*, p 124, 153
- ⁷Hopkins and Cole Jour Physiol, 1902, **xxvii**, 418
- ⁸Benedict, S R Jour Biol Chem, 1909, **vi**, 51
- ⁹Lang Jour Biol Chem, **lxix**, 397

ON THE CULTURAL CHARACTERISTICS OF LACTOBACILLUS ACIDOPHILUS*

By C. ROOS† PHILADELPHIA PA

AS A starting point for this discussion reference can be made to the work of Cruickshank and Berry¹ on the presence of *Bacillus acidophilus* in human feces, reported in the British Medical Journal of November, 1924. The investigation referred to was made at the instance of the British Medical Council. The report dealt with the types of acidophilus bacilli found in fecal specimens from adults and children. As many as ten different strains were isolated from a single specimen. No relation between morphology, colony formation, sugar or milk reactions could be established, and the conclusion was reached that the term *L. acidophilus* covered a considerable group of organisms.

These conclusions of Cruickshank and Berry are generally in agreement with the reports of the earlier observers. Moro² in 1900 described the *L. acidophilus* surface colonies as having a delicate hair-like periphery, denser towards the center, the deeper lying colonies showing the characteristic branching threads. Frequently one can see colonies of other forms without threads.

Some of our recent contributors have taken a very limited view in regard to the diagnostic cultural characteristics of *L. acidophilus*. Rettger³ and his coworkers are inclined to regard as *L. acidophilus* only those strains that form *L. bulgaricus*-like colonies on agar, ferment certain carbohydrates and produce only a mild acidity in milk. A typical acidophilus colony according to our recent digest galactose agar is one with fuzzy edges (when observed under the low power objective) and has been designated as Type x, and one showing only a very few hair-like projections designated as Type y. They would consider all other strains as unclassified intermediates. Kulp⁴ has recently reaffirmed the earlier conclusions reached by Rettger and himself and defines the typical *L. acidophilus* as a Gram positive nonmotile rod which produces a mild acidity of 0.7 to 0.8 per cent in sterile milk employing twenty-four hours incubation at 37° C.

An acid culture of this degree and the flavor are of importance in the production of a palatable acidophilus milk but the acid production is hardly suitable as a basis for classification since the acidity produced varies with the amount of inoculum and the vigor of the strain. Moreover the freshly isolated strains from the intestinal tract show the greatest variations in acid production in milk. Milk is coagulated by some strains in two days or even less others may not coagulate milk at all. Coagulation generally is in pro-

*Read at the Annual Meeting of the Society of American Bacteriologists Philadelphia December 8, 1926.

†H. I. Mulford Co. Biological Laboratories Philadelphia

portion to growth rapidity and vigor of strain, regardless of the type of colony formed

The colony most commonly met with in fecal specimens is of the solid type. The cells forming the solid colonies are far more resistant to deleteri-

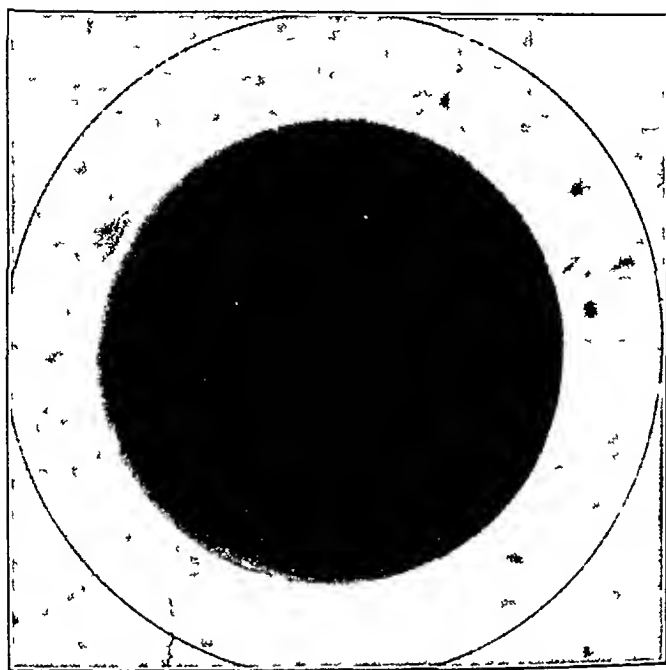


Fig 1—*L. acidophilus* variant 1 Surface colony two days old

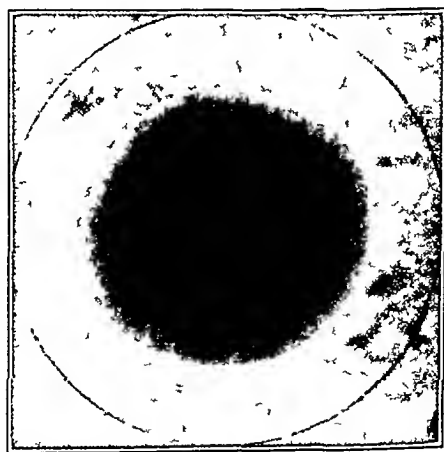


Fig 2—*L. acidophilus* variant 2 Surface colony two days old

ous influences, both inside and outside the intestinal tract. They never disappear completely from the intestines, and on milk or carbohydrate diet they increase greatly in numbers. In some individuals the strains that produce solid colonies predominate persistently over all other types.

The filamentous type is most commonly obtained from fecal specimens

from individuals on milk or high carbohydrate diet. When individuals, showing no filamentous type colonies, are placed on milk diet or fed lactose over long periods the filamentous type colonies appear. Whence do they come?



Fig. 3—*L. acidophilus*, variant 1. Surface colony, twenty-four hours old.

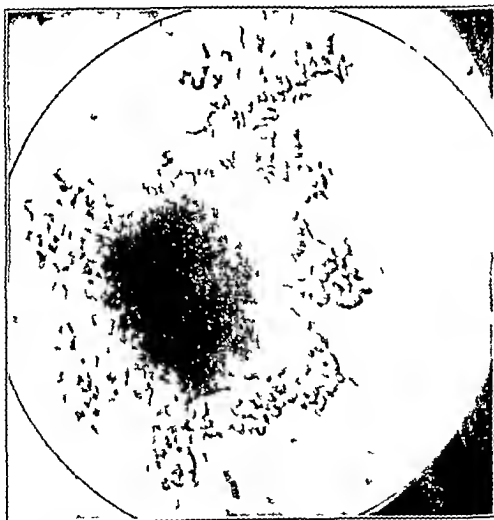


Fig. 4—*L. acidophilus* variant 3. Surface colony, twenty-four hours old.

Do they evolve from the solid type of those within as a result of the diet or are they introduced from outside?

Our cultural findings, using acid milk trypsin digest agar media of the acidophilic bacilli in human intestines have given us results comparable to

those reported by Cruickshank and Berry. On the basis of colony formation we have isolated as many as five distinct strains from one specimen. These strains all grew on media of such acidity (P_H 5.0) as would inhibit the growth of other organisms except some yeasts and cocci.

Our interest in *L. bulgaricus* and *L. acidophilus* preparations for therapeutic purposes dates back to 1913, and during this period, there have come under our observation many strains of *L. acidophilus* from authentic sources. All, except two, form *L. bulgaricus*-like colonies. One of these two has proved to be an especially vigorous strain, and is less fastidious in regard to its cultural requirements. This strain was isolated by us four years ago, from an acidophilus milk. Upon repeated platings on whey agar, the surface colonies were uniformly round and convex, enterococcus like. The deep colonies were

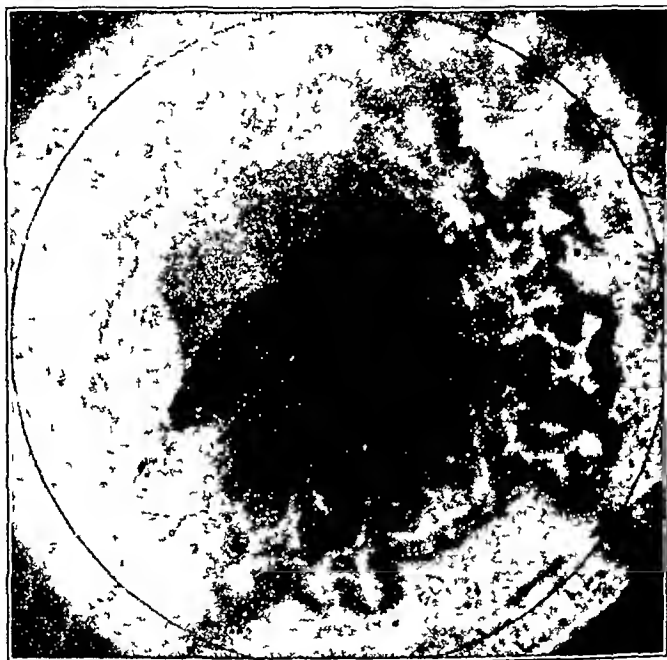


Fig 5—*L. acidophilus* variant 3. Surface colony five days old.

mostly beech-nut-like. Some were elliptical. In liquid media typical *L. acidophilus* growth occurred. A finely flocculent sediment formed with growth adhering to the sides, leaving the supernatant clear. Stained smears showed Gram positive rods, uniform in size, single or in chains.

For purposes of transformation of the intestinal flora, this strain proved highly efficacious, but on account of its cultural characteristics some authorities regard it as atypical.

We consulted three of the seven members constituting, at present, the Committee on Lactic-Acid Ferment Preparations for the American Council on Pharmacy and Chemistry. Our first consultant informed us that it would not be practical to admit this strain as true *L. acidophilus* on account of the atypical colony. The second consultant stated that he had never seen an acidophilus like it, and doubted its authenticity.

The third consultant after a brief examination of our culture pronounced it as impure consisting of three different strains. Upon closer examination he found that these strains were variants. Furthermore, he found that these variants differed in their power of carbohydrate fermentation. Fermentation tests made in duplicate gave the following results. Variant (1) negative to maltose, sucrose, unheated levulose, variant (2) positive to maltose, weakly positive to sucrose, negative to unheated levulose, variant (3) negative to maltose and sucrose, weakly positive to unheated levulose. Surface tension tests showed that this strain in common with other *L. acidophilus* strains, grows at 39 dynes whereas *L. bulgaricus* does not grow below 42 dynes.

This culture of *L. acidophilus* has been carried along in sterile milk since its isolation four years ago. From this the following distinct types of colonies have been obtained through repeated selections. (1) an enterococcus like colony, round, dense and smooth Fig. 1. (2) streptococcus like col-



Fig. 6—*L. acidophilus* variant 3. Colony 8 days old.

ony, the edges somewhat irregular, surface elevation raised and slightly umbonate, Fig. 2, (3) *L. bulgaricus* like colony. Figs. 3, 4, 5 and 6. This latter colony, when about fifteen hours old does not differ from *L. bulgaricus* or *L. acidophilus* Type α colony. All these colonies when grown on milk trypsin digest medium, remain true to type. Single colony fishings of any of these types placed in milk tend to produce cell types, some of which form the filamentous colonies when plated.

The filamentous colonies contain many longer rods, which show more tendency towards pleomorphism. The explanation seems tenable that the shorter rods of uniform size growing in palisade formation or in tortuous chains evolve solid or slightly irregular colonies whereas the longer rods result in hair like projections such as is characteristic of *L. bulgaricus*. The evolution of these colony types can be readily observed under the microscope on casein digest agar plates. Our observations are not confined to the above strain. Similar occurrences have been noted in cultures of other types. One of these cultures is a single cell isolation.

CONCLUSIONS

From our observations we conclude that the name *L. acidophilus* should be applied to a group of biologically related strains, variable in cultural and morphologic characteristics

We regard the various types of strains of lactobacilli found in the intestinal tract as variants

Acid production, carbohydrate fermentation, colony formation are variable characteristics and alone do not constitute basis for classification. Nor do any of these characteristics serve as reliable indications of therapeutic potency. From the standpoint of clinical usage, source of the strain, its nutrient and physical requirements to facilitate growth, and temperature range are important factors and must necessarily be considered.

We believe that the presence of acidophilic bacilli in the intestinal tract of man is natural and beneficial. Culturally identical strains may show distinct immunologic differences, depending on human or animal sources, indicating a change due to adaptation to a particular environment. Therefore, it seems logical to use for therapeutic purposes only strains of *L. acidophilus* isolated from normal human sources.

Such a strain should be vigorous, not fastidious as to nutrient requirements, little sensitive to oxygen tension, capable of growth in a medium of varied surface tension, and with a wide temperature range. Such is the strain of choice, provided it meets the crucial test of proved therapeutic value.

Grateful acknowledgement is herewith made to Dr. John Reichel for helpful criticism and important suggestions.

REFERENCES

- ¹Cruckshank, J., and Berry, D. W. Brit. Med. Jour., Nov. 22, 1922, No. 3334, p. 944
- ²Moro, Ernst. Jahrb. f. Kinderh., 1900, 38
- ³Kulp, Walter L., and Rettger, Leo F. Jour. Bacteriol., July, 1924, 12, 357
- ⁴Kulp, Walter L. Jour. Am. Med. Assn., Sept. 11, 1926, LXXVII, 833

ACETONE BODY FORMATION AND THE CHEMICAL AFFINITY OF OXYGEN FOR CARBOHYDRATE AND FATTY ACID*

BY DWIGHT M. ERVIN M.D. SAN FRANCISCO, CALIF.

IN A previous article it was shown mathematically that the blood sugar curve and the excretion of glucose in the urine of a diabetic could not be explained upon the basis of failure to oxidize the glucose.¹ The purpose of this paper is an explanation of the chemistry of the 'acetone bodies' found in the same pathology.

Fats and carbohydrates combine with oxygen in the work of the body. The body is a closed system of oxidation. Work is done (energy change) in the oxidation of each by a given quantity of oxygen held within a narrow limit of constancy.

Let W_1 = work done in oxidizing the fatty acid

Let W_2 = work done in oxidizing the lactic acid

Hence the ratio of oxygen taken by each is the ratio of the work done in the oxidation of each

If a = quantity of oxygen liberated from hemoglobin

x = oxygen to fatty acid

$a - x$ = oxygen to lactic acid

$$\text{Hence } \frac{x}{a - x} = \frac{W_1}{W_2} = \frac{\text{chemical affinity of O for fatty acid}}{\text{chemical affinity of O for lactic acid}}$$

$$\frac{x}{a - x} = \frac{RT \log K_1 - RT \sum v \log C_1}{RT \log K_2 - RT \sum v \log C_2}$$

K_2 = Equilibrium constant of oxygen and lactic acid †

K_1 = Equilibrium constant of oxygen and fatty acid †

The distribution of oxygen between the fatty acid and lactic acid is determined by their chemical affinities.

It is the intention of this work to examine the above equation for an explanation of the formation of the 'acetone bodies' that will be free of the necessity of the oxidation of glucose or one of its derivatives, free of that expression 'fats burn only in the fire of carbohydrates'.

As recovery of the contracted muscle takes place energy equal to that given up on contraction must be returned to the muscle and this energy is supplied by the oxidation of the fatty acids and carbohydrates (or its derivative). The 'foods' are delivered by some mechanism that determines their ratio, to a given quantity of oxygen released from the hemoglobin system. The quantity of oxygen released is held within narrow limits to a constant quantity. The equation above will give the distribution of this oxygen between the carbohydrate and the fatty acid to be burned.

The quantity of work done by the carbohydrate (to be referred to as lactic acid for this is the probable form in which it is oxidized) and by the

Received for publication January 1927

†These equilibrium constants are now being determined

fatty acid in restoring the muscle from a state of dissipated energy to its initial state will depend upon what part of the oxygen released from the hemoglobin each receives. From the equation above by keeping the oxygen quantity constant and altering the ratio of fatty acid to the lactic acid intermediate products of the fatty acid will be produced including the "acetone bodies". To this distribution the ratio of their respective affinities for the oxygen applies

$$\begin{aligned} W_1 &= R T \log K_1 - R T \sum v \log C_i \\ W &= R T \log K_2 - R T \sum v \log C \\ C_i &= \text{equal the initial concentration of fatty acid reactants and oxygen} \\ C &= \text{equal the initial concentration of lactic acid reactants and oxygen} \end{aligned}$$

$$\frac{W_1}{W} = \frac{R T \log K_1 - R T \sum v \log C_i}{R T \log K_2 - R T \sum v \log C}$$

Since in the mixture the CO, acetic acid, and oxygen are common to both this may be written—

$$\frac{e^{\frac{W_1}{RT}}}{e^{\frac{W_2}{RT}}} = \frac{\frac{K_1}{C_i}}{\frac{K_2}{C_2}}$$

Cancelling the reactant and resultants common to both, the equation becomes—

$$\begin{aligned} \frac{e^{\frac{W_1}{RT}}}{e^{\frac{W_2}{RT}}} &= \frac{K_1 \times \text{concentration of fatty acid}}{K_2 \times \text{concentration of lactic acid}} \\ \frac{W_1 - W_2}{R T} &= \log \frac{K_1 \times \text{concentration of fatty acid}}{K_2 \times \text{concentration of lactic acid}} \end{aligned}$$

$W_1 = Q_1 a$ where Q_1 is an operator that converts the grams of oxygen used into calories when the oxygen is combining with the fatty acid, and

Q_2 = that which converts the oxygen into calories when combining with lactic acid*

$W = Q(a - v)$ for lactic acid combustion

Substituting the equation becomes in terms of oxygen distribution instead of work terms—

$$Q_1 v - Q_2 (a - v) = R T \log \frac{K_1 \times \text{concentration of fatty acid}}{K_2 \times \text{concentration of lactic acid}}$$

From this equation it is seen that the affinity of each substance sharing the oxygen is measured by the equilibrium constant and the concentration. If the oxygen quantity be maintained constant and insufficient to completely oxidize both there will be formed intermediate products of the substance highest in concentration.

This equation is tested in the following manner for the explanation of the "acetone bodies"

The oxidizing agent used was potassium dichromate and hydrochloric acid. This mixture with either lactic or B-hydroxy butyric acid would come to an equilibrium of carbon dioxide pressure within twenty-four hours.

The method of analysis of B-hydroxy butyric acid and acetone (aceto

*From the work of F. G. Benedict and E. L. Fox in Jour. Biol. Chem. 1911 783 both Q_1 and Q_2 will have the same value (10.8 per m. mol. of oxygen for the small calorie)

acetic acid considered as acetone) was that of Van Slyke's with certain precautions to be discussed later

The method of oxidation was in the cold over a period of twenty four hours. Both Shaffer³ and Van Slyke⁴ state that in the cold the oxidation of β -hydroxy butyric acid proceeded in some other way than through acetone. This from the figures of Van Slyke seems apparent unless another solution for Van Slyke's figures can be offered.

It is possible that the oxidation of β -hydroxy butyric acid proceeds in both heat and cold through acetone but in the method of Van Slyke the acetone as formed is caught in the insoluble compound—mercury sulphate—and removed from the field of oxidation while in the cold the acetone is oxidized through to acetic and formic acid.

TABLE I

	K ₂ Cr ₂ O ₇	HCl	Boil	ACETONE YIELD
4 hr in cold	50 mg	50 ml	50 ml	0.6 mg
Heated in reflux 1½ hr with dichromate and acid H ₂ SO ₄ added	50 ml	50 ml	50 mg	0.5 mg
Dichromate added after boiling in H ₂ SO ₄ had begun	50 ml	50 ml	50 mg	22.0 mg

TABLE II

	K ₂ Cr ₂ O ₇	HCl	Boil	ACETONE YIELD
4 hr in cold	50 ml		50 ml	13.5 mg
Heated in reflux 1½ hr with dichromate and acid. Then H ₂ SO ₄ solution added	250 ml		50 ml	14.0 mg
Dichromate added after boiling in H ₂ SO ₄ had begun	250 ml		50 ml	23.1 mg

*The sulphuric acid dichromate mixture of Van Slyke was then tried in experiment II of Kahlbaum (d) β -hydroxy butyric acid.

These two experiments were good evidence that both in the heat and cold the oxidation of β -hydroxy butyric acid proceeded through acetone but in the technique of Van Slyke 0.75 mol acetone per mol of β -hydroxy butyric acid was caught in the insoluble compound and removed from the oxidation field while 0.25 of acetone slipped through to acetic and formic acid because of the speed of the reaction.

Van Slyke found that when he increased his initial reactants chromate and sulphuric acid he did not recover 0.75 mol per mol of the fatty acid. When he increased his reactant he increased the speed of the reaction and more acetone would pass through to acetic and formic acid.

By increasing the acids the speed of the reaction is increased and a smaller yield of acetone will result unless the acid be the end product of the fatty acid oxidation. We may decrease the driving force of a reaction by adding one of the final derivatives of oxidized acetone. By adding acetic acid to the oxidation of β -hydroxy butyric acid in Van Slyke's technique we may slow the reaction of acetone to acetic acid and increase the yield of acetone if it is a question of the 0.25 mol slipping by the insoluble mercury compound, but not if the direction is some other than through acetone.

TABLE III

O	BoH	HCl	ACETIC ACID	ACETONE	YIELD PER MOL
73 mg	80 mg	0 mg	0 mg	33.5 mg	0.75 mg
73 mg	80 mg	0 mg	200 mg	40.5 mg	0.90 mg
73 mg	80 mg	850 mg	0 mg	22.2 mg	0.50 mg

TABLE IV

O	BoH	HCl	ACETIC ACID	ACETONE	YIELD PER MOL
75 mg	75 mg	0 mg	0 mg	30.4 mg	0.74 mg
73 mg	75 mg	0 mg	200 mg	37.5 mg	0.91 mg
73 mg	75 mg	850 mg	0 mg	21.8 mg	0.49 mg

Outside of the acetic acid added to the B-hydroxy butyric acid before placing it in the reflux the technique was identical to Van Slyke's*.

The presence of acetic acid, which is not oxidized by the dichromate mixture, was sufficient to retard the reaction to give a yield of 0.90 mol of acetone per mol of B-hydroxy butyric acid.

This action of acetic acid puts beyond doubt that the oxidation of the fatty acids proceeds through acetone in both heat and cold.

All other acids decrease the yield. Sulphuric acid gives a yield of 0.75 mol while the hydrochloric acid mixture yields 0.50 mol. Throughout the following experiment where the B-hydroxy butyric acid is computed from acetone when oxidized by the hydrochloric acid it is computed as one mol of the fatty acid to 0.5 mol of acetone.

TABLE V

OXYGEN	BoH	H ₂ SO ₄	HCl	ACETONE	YIELD
73 mg	80 mg	Van Slyke	0 mg	33.5 mg	0.75 mol
73 mg	80 mg		850 mg	22.2 mg	0.50 mol
73 mg	80 mg		850 mg	21.8 mg	0.49 mol

The technique of the following experiments is: The required amounts of B-hydroxy butyric acid with potassium dichromate to yield 73 mg of oxygen and 825 mg of hydrochloric acid were placed in a 50 cc flask and made up to 15 cc in final volume. The flasks were set aside to oxidize for twenty-four hours at room temperature. At the end of this time except in the low concentrations of B-hydroxy butyric acid the clear green of the chromic acid was distinct showing that the oxidation was complete. The mixture of B-hydroxy butyric acid, lactic acid, acetone, and other derivatives was then placed in a 500 cc flask with 290 cc of Van Slyke's mercury sulphate acid mixture and boiled for one and one-half hours under a reflux. To this reflux was always attached a condensing tube which dipped below 30 cc of cold water. At the end of one-half hour this water was placed through the reflux condenser into the boiling flask. This was taken as a precaution to prevent any possible loss of acetone. At the end of one and one-half hours the solution was cooled and filtered through an alundum crucible. Since no other dichromate was added during this boiling the quantity of acetone formed in the distribution of the initial concentration of oxygen between lactic acid

*Acetic acid does not yield a precipitate with Van Slyke's reagents.

and B hydroxy butyric acid would be given. The crucible was placed in a drying oven at 110°C for one hour.

The filtrate was returned to the reflux apparatus and when brought again to boiling 10 cc of 5 per cent dichromate was added. When 320 mg B hydroxy butyric acid was used 15 cc of 5 per cent dichromate solution was used. At the end of one and one half hours boiling the solution was filtered through a crucible and dried in the drying oven. This gave the B hydroxy butyric acid not oxidized by the initial 73 mg. From these experiments could be deduced the quantity of oxygen taken by the fatty acid.

If the oxygen remained insufficient to completely oxidize the mixture of lactic acid and B hydroxy butyric acid then as the concentration of the B hydroxy butyric acid increases from Van't Hoff's isotherm it can be reasoned that more oxygen will be taken by the B hydroxy butyric acid in total but less per mol which will cause more B hydroxy butyric acid to be attacked by the oxygen but less completely burned.

TABLE VI
INCREASE OF B HYDROXY BUTYRIC ACID

O	BOH	LACTIC	ACETONE	BOH RECOVERED	TOTAL O TO BOH	O PER MOL BOH	BOH OXIDIZED COMPLETELY
13 mg	40 mg	45 mg	0.0 mg	0.0 mg	24.6 mg	63.0 mg	40.0 mg
13 mg	53 mg	45 mg	4.5 mg	1.79 mg	27.7 mg	54.8 mg	43.2 mg
13 mg	90 mg	45 mg	5.5 mg	1.79 mg	32.9 mg	42 mg	51.3 mg
13 mg	120 mg	45 mg	10.0 mg	4.58 mg	37.0 mg	36.0 mg	58.3 mg
73 mg	160 mg	45 mg	14.5 mg	7.16 mg	42.5 mg	27.6 mg	62.0 mg
73 mg	320 mg	45 mg	25.0 mg	18.0 mg	44.5 mg	23.9 mg	93.8 mg
73 mg	160 mg	0 mg	2.2 mg	35.9 mg	13.0 mg		110.3 mg

TABLE VII

O	BOH	LACTIC	ACETONE	BOH RECOVERED	TOTAL O TO BOH	O PER MOL BOH	BOH OXIDIZED COMPLETELY
13 mg	80 mg	0 mg	0.5 mg	0.0 mg			
73 mg	80 mg	45 mg	5.5 mg	7.2 mg	16.0 mg		
73 mg	80 mg	180 mg	7.0 mg	23.9 mg	0.0 mg		
73 mg	80 mg						
73 mg	160 mg	0 mg	2.2 mg	35.8 mg	7.0 mg		119.3 mg
73 mg	160 mg	15 mg	14.5 mg	7.16 mg	42.5 mg		62.5 mg
73 mg	160 mg	155 mg	1.5 mg	1.47 mg	10.6 mg		10.6 mg
13 mg	160 mg	80 mg	17.5 mg				

Large quantities of lactic acid yield a precipitate with Van Slyke's reagents

With a mixture of lactic acid and B hydroxy butyric acid to be oxidized by a constant quantity of oxygen we may by increasing the concentration of the B hydroxy butyric acid theoretically produce a system in which all the B hydroxy butyric acid has been oxidized to acetone. But as the acetone is formed a new distribution takes place. The oxygen is now distributed between the two acids and acetone.

Table VI shows the distribution of oxygen in such a system. Chiefly that as the B hydroxy butyric acid is increased more oxygen is taken by the B hydroxy butyric acid, but less per mol with a consequent increase of acetone.

In a similar manner there is an increase of acetone when the concentration of lactic acid is increased. Here, however, the total quantity of oxygen taken by the B-hydroxy butyric acid as well as the oxygen per mol is decreased.

It is interesting to note in the passing that when B-hydroxy butyric acid is only slightly above the quantity completely oxidized by a given quantity of oxygen, the ratio $\frac{\text{Acetone}}{\text{B-hydroxy butyric (unburned)}}$ became greater than one, but as the acid increases in concentration the ratio drops rapidly. This is to be expected from the equation. When the B-hydroxy butyric acid increases to a quantity where all the oxygen taken by the acid is less than mol for mol the B-hydroxy butyric acid will rise to about the acetone output.

Hubbard and Wright⁷ noticed this discrepancy and ascribed it to the fact that there was a differential secretion of the kidney. This seems rather impossible when we find the same changing ratio in the blood.⁸

From Hubbard and Wright⁸ and Joslin⁹ are added some tables showing the ratio of acetone to the B-hydroxy butyric acid.

TABLE VIII

	ACETONE	B-HYDROXY BUTYRIC
	0.25 mg	0.17 mg
	0.59 mg	0.53 mg
	0.89 mg	1.35 mg
	1.44 mg	4.27 mg
	2.17 mg	5.27 mg

TABLE IX*

DAY	ACETONE	B-HYDROXY BUTYRIC
1	0.31 mg	0.0 mg
2	1.50 mg	2.90 mg
3	1.67 mg	17.94 mg
4	3.54 mg	18.47 mg
15	0.0 mg	0.0 mg
16	0.02 mg	0.0 mg
17	1.20 mg	0.17 mg
18	1.52 mg	5.44 mg
19	1.55 mg	13.54 mg

* Again from Joslin Table 133

SUMMARY

An explanation of the "acetone bodies" formation based upon the theory that work done in the recovery of the system is obtained by the oxidation of fatty acids and glucose or its derivative.

With the theory that work is done by the oxidation of these two the distribution of oxygen between the two is then developed by Van't Hoff's isotherm, or equation for chemical affinity.

This chemical affinity of oxygen for fatty acid and lactic acid is a function of their equilibrium point and their concentration. The distribution of oxygen in oxidizing lactic acid and fatty acid is dependent upon the concentration of the lactic acid and the fatty acid.

When the concentration of oxygen is maintained constant and the concentration of the fatty acid increased to the degree that there is insufficient oxygen to completely oxidize the fatty acid there will result the greater quantity of oxygen will be distributed to the fatty acid (decreased R Q) but a decreased quantity of oxygen per mol of fatty acid (increased intermediate derivatives of oxidation acetone bodies)

The ratio $\frac{\text{acetone}}{\text{B hydroxy butyric}}$ is a linear equation of the quantity of oxygen, the quantity of B hydroxy butyric acid and the chemical affinity of oxygen for the two

The ratio when small quantities are excreted in the urine is greater than one, when large quantities are excreted drops rapidly below one. This condition is paralleled in the above equation in the test tube

The explanation offered for the chemistry of the "acetone bodies" is independent of the theory of the oxidation of glucose (lactic acid) and is applicable to all pathologic states where the quantity of fatty acid to be oxidized rises above a given ratio of oxygen per mol of fatty acid

REFERENCE

- *Ervin, D M Jour Lab and Clin Med 1917 xxxi 18
- *Van Slyke D D Jour Biol Chem 1917 xxxv 411
- *Shaffer, P A Jour Biol Chem 1908 99 1-211
- *Van Slyke, D D Jour Biol Chem 1917 xxxv 41
- *Van Slyke, D D Jour Biol Chem 1917 xxxv 4
- *Van Slyke, D D Jour Biol Chem 1917 xxxv 408
- *Hubbard and Wright Jour Biol Chem 1917 1400
- *Hubbard and Wright Jour Biol Chem 1917 1418
- *Joslin New York, 1923 p 106

SPONTANEOUS DEATH IN A RABBIT WITH THE ISOLATION OF A TYPE IV PNEUMOCOCCUS

By EVERETT S. SANDERSON, PH.D. CHARLOTTESVILLE, VA

WHILE the small laboratory animals are susceptible to injections of pneumococci, rarely do these organisms seem to play a role in natural infections. Keegan¹ found them as secondary invaders in an epidemic among mice and guinea pigs caused by *B. bronchisepticus* but the present isolated case is of interest in that an organism of this type was apparently the cause of spontaneous death.

The animal in question was from laboratory stock and in November had been injected intravenously and intraperitoneally with an old stock culture of anthrax for demonstration to students. Insofar as outward appearances were concerned however, the injections had no ill effects, and about the middle of December the rabbit was housed with a normal one and the two kept in the main laboratory. The former animal was quite thin, but always

¹From the Department of Pathology and Bacteriology, University of Virginia.
Received for publication April 30, 1918.

active and eager for food, and at no time was there any indication of ill health. On the morning of January 27 it was found dead, the belief being that it had suddenly succumbed to latent anthrax. That this was not the case is evident from the following observations made at autopsy.

The peritoneal cavity contained a small amount of slightly reddish fluid, while the pericardial sac was filled with a clear serous fluid. The intestines and stomach were distended with gas, the spleen was not enlarged, and the gall bladder contained a yellowish, granular pasty material. The lungs were pale and not enlarged. Smears from the heart's blood showed vast numbers of Gram-positive, lancet-shaped diplococci possessing a distinct halo. A similar picture was observed in a smear from the liver. The pericardial fluid contained smaller numbers of diplococci and was free from cellular elements. A few macrophages were to be observed in the peritoneal fluid and the diplococci were numerous although less so than in the heart's blood. The organisms were extra cellular. In all these smears none but lancet diplococci were seen. Smears from the material in the gall bladder, however, showed great numbers of Gram-negative coli-form bacilli. The lancet diplococcus was isolated in pure culture from the spleen and peritoneal fluid, while in the heart's blood and pericardial fluid it was mixed with a colon bacillus of the type producing acid and gas from saccharose. This latter organism was obtained in pure culture from the gall bladder. No anthrax bacilli were found.

Five-tenths cc of a bouillon culture from the spleen injected intra peritoneally into a mouse caused death in twenty-four hours. The organisms in the exudate were numerous, soluble in ox-bile but failed to agglutinate with standard type sera. They were recovered from the heart's blood. Culturally the organisms produced acid and clot in milk serum water and the colonies on blood agar were umbilical with formation of a slight amount of green pigment. These characteristics would indicate the organism to be a Type IV pneumococcus.

The finding of innumerable pneumococci in the smears of the heart's blood of this case, together with similar organisms in pericardial and peritoneal fluids, would lead one to suggest that the animal had succumbed to a pneumococcus septicemia. As was mentioned above the two rabbits had been housed in a room daily occupied by students and so it might be reasonable to assume that the infection was air borne. To ascertain whether the companion rabbit was also harboring this organism, cultures were made from the anterior nares and pharynx. Pneumococci were not recovered and a cardiac blood culture remained sterile after eight days' incubation.

SUMMARY AND CONCLUSIONS

A spontaneous death in a stock rabbit with isolation of a Type IV pneumococcus from the heart's blood, spleen, peritoneal and pericardial fluids has been described. Microscopic pictures from smears of heart's blood and tissues would indicate a pneumococcus septicemia.

REFERENCE

A STUDY OF BILE SECRETION FROM A CASE OF BILIARY FISTULA*

By S GORDON ROSS M B (TOR) PASADENA CALIF

THE bile secretion in a case with biliary fistula was studied to determine the amount and uniformity of the daily secretion and the effect thereon of ingested bile and various diets. This study was made in conjunction with Dr Harry S McGee

CASE REPORT

D V R male aged 56 years height 60 inches weight 110 pounds was admitted to the Pasadena Hospital August 14 1925 in a deeply jaundiced and cachectic condition. He gave a history of two operations. The first was for excision of a gastric ulcer in 1918

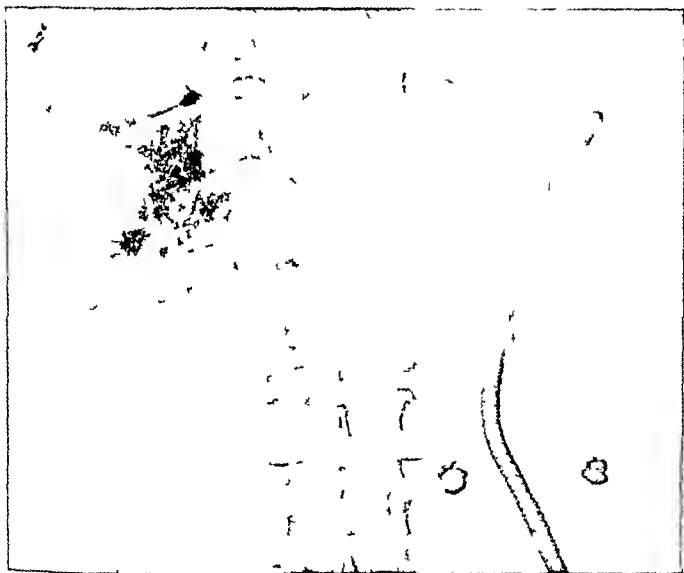


Fig 1—Immediately before injection of lipiodol

necessitated by severe hemorrhage. In November 1924 an acute intestinal obstruction was relieved by loosening adhesions around the upper small intestine. Jaundice developed after this operation and became progressively deeper until his skin was deeply bronzed. At opera-

From Metabolic Department Pasadena Hospital
Received for publication April, 18 1926

tion on August 24, 1925, for this condition, the abdominal cavity was found to contain a great many adhesions in the upper right quadrant. The common bile duct was so enlarged as to resemble the small bowel. On opening it, a thorough exploration revealed no stones, either in it or in the hepatic ducts. The Ampulla of Vater was found embedded in a mass of dense fibrous tissue. A fine probe could not be introduced into the duodenum, through the Ampulla. A catheter was inserted in the duct as a drain. The gall bladder could not be found.

The wound healed tightly around the catheter allowing no loss of bile. The patient developed a desire to drink his own bile which he took, as required, from a bottle held by a belt around his waist.

Our studies on this case started in April, 1926. At this time the jaundice had completely disappeared. He weighed 140 lbs and was able to carry on light work. His admission into the hospital at this time was solely for the purpose of this study, as he otherwise was apparently normal.



Fig. 2—Immediately after injecting lipiodol

In studying biliary fistulae, it is of the utmost importance to rule out the presence of a functioning gall bladder. In addition to the evidence obtained from the bile itself, we have an operative report that no gall bladder was found. The biliary tract was further investigated by injecting lipiodol into the drainage tube with the patient in Trendelenberg's position. X-ray plates were made by Dr. C. H. Parker, before injection, just after injection and three days after injection. No gall bladder outline could be seen, although the biliary radicles were very well outlined.

Daily Amount—The twenty-four hour bile secretion varied from 725 to 1010 cc, the average being 863 cc. The average twelve-hour day specimen contained 523 cc and the corresponding night specimen contained 340 cc. These figures were obtained during a period when he ingested about 700 cc of bile daily. On discontinuing the bile ingestion, the 24-hour secretion decreased to an average of 556 cc ranging from 505 to 725 cc. This is shown clearly in the accompanying graph, as well as in Table I.

TABLE I

BILE INTAKE	BILE SECRETION					
	DAY		NIGHT	TOTAL DAY		
CC. PER 24 HOURS	8 A M	8 P M	8 P M 8 A M	LOW	HIGH	AVERAGE
Average 700 cc	523		340	725	1010	863
No intake	360		55	05	725	556

Daily Uniformity of Secretion—The daily specimen was collected at two hour intervals in order to determine if daily maxima occurred in the secretion. It was found that for two hour periods during the day the amounts were remarkably uniform.

8 A M	10 A M	8	10
10 A M	12 M	8	
12 M	2 P M	8	
2 P M	4 P M	8	
4 P M	6 P M	8	
6 P M	8 P M	8	

Fig. 3.—Three days after beginning bile

Specific Gravity—During bile ingestion the specific gravity varied between 1.008 and 1.012. When no bile was taken the specific gravity dropped to 1.006 and 1.010. This is in accordance with the finding of Wisner and Whipple.

The fact that the bile was continually about 1 010 specific gravity suggests strongly that we were dealing with bile secreted directly from the liver, and uninfluenced by the concentrating function of the gall bladder. Gall bladder bile and biliary fistula bile are differentiated in Hawk². The former usually has a specific gravity of about 1 040.

Diet—During the first ten days of the sixty-day period of observation, a regular hospital diet was given. Fat, 90 gm, protein, 95 gm, carbohydrate, 280 gm, calories, 2310. The average daily bile secretion was 860 cc. A lower caloric diet was used then with the protein increased. Carbohydrate, 86, protein, 134, fat, 96, calories, 1744. The secretion on this diet diminished to 780 cc. It was poorly tolerated by the patient. It appeared that when he complained of the food, the bile secretion diminished. His intolerance for fats made it impossible to study a high fat diet. Carbohydrates, especially fruit juices and glucose, were tolerated well by the patient. A diet of carbohydrate, 450 gm, protein 40 gm, fat, 20 gm, calories, 2140, was given. This diet was very acceptable to him, and resulted in an average twenty-four hour secretion of 900 cc.

Blood Chemistry—During a high carbohydrate diet that was well tolerated, the blood chemistry showed

Nonprotein nitrogen	35.1 mg per 100 cc blood
Uric acid	2.1 mg per 100 cc blood
Creatinine	2.0 mg per 100 cc blood
Sugar	100.0 mg per 100 cc blood
Chlorides	430.0 mg per 100 cc blood
CO ₂ Vol per cent	80 per cent

These readings are within normal limits.

During a high protein diet, the blood chemistry showed

Nonprotein nitrogen	46.2 mg per 100 cc blood
Uric acid	4.7 mg per 100 cc blood
Sugar	154.0 mg per 100 cc blood
Chlorides	460.0 mg per 100 cc blood
CO ₂ Vol per cent	45 per cent

which we interpret as a moderate retention of protein catabolites accompanied by moderate acidosis.

Special interest was taken in studying the blood calcium and phosphorus, because of the changes noticed in blood clotting time in jaundiced cases, and the osteoporosis that sometimes results in cases of complete biliary fistula of long standing. The blood calcium and phosphorus were determined several times during the periods of bile ingestion as well as the period when no bile was ingested, and they were found to remain constant,—calcium, 12.0 mg per 100 cc blood, and phosphorus, 4.5 mg per 100 cc blood.

SUMMARY

1 Bile secretion varied from 725 cc to 1010 cc per twenty-four hours during bile ingestion.

2 Bile secretion varied from 505 cc to 725 cc per twenty-four hours during no bile ingestion.

3 Bile was secreted at a constant rate during the day. The secretion tended to diminish during the night.

4 The quantity and specific gravity of the bile were both increased by bile ingestion.

5 High carbohydrate diet was best tolerated. High fat diet could not be tolerated. High protein diet resulted in nonprotein nitrogen retention, accompanied by moderate acidosis.

6 Blood calcium and phosphorus remained constant and independent of bile ingestion.

7 Lipiodol may be used to investigate a biliary fistula and the biliary radicles.

REFERENCES

- ¹Wisner, F. P., and Whipple, G. H. *Am Jour Physiol* March 1922 lx No 1
Hank Philip B. *Practical Physiological Chemistry* ed 8 Philadelphia 1923 page 206

BARBITURIC ACID DERIVATIVES AS ANESTHETICS AND METHODS FOR ADMINISTRATION*

By J. FRANK PEARCY AND M. M. WEAVER

IN 1922 Tatum and Parsons¹ described in this journal the use of barbital as an anesthetic for dogs. Since that time other derivatives of barbituric acid have been studied as hypnotics and have been placed upon the market for their clinical use. Having found barbital very useful in our experiments on secretion and bulbospinal reflexes² we thought it profitable to study the newer preparations as anesthetics for animals.

Tatum and Parsons described only the per os administration of barbital sodium for anesthesia in animals. This has been the method in general use. Although it is the most physiologic method of administration other methods are practically much superior. Believing that the little use which is being made of this valuable anesthetic is due to unfamiliarity with easy and certain methods of administration we think it timely to describe them in some detail.

Per Os Method—The ordinary stomach tube method is used. It is well to provide that the animal shall not have been fed the day it is to be anesthetized because of uncertainty of absorption and danger of vomiting. If for a minute or two following withdrawal of the stomach tube the attendant holds the animal's head up and administers a friendly pat or two the possibility of vomiting is made negligible, that is at least if one has not introduced too much water into the stomach.

This method of administration although the most physiologic has serious

From the Departments of Physiology of the University of Chicago and West Virginia University.

Received for publication May 30 1927

drawbacks If any of the solution is vomited the animal usually has to be discarded for several days, as one cannot readily replace the lost drug with any accuracy The time required for surgical anesthesia is variable due to variations in gastrointestinal motility and absorption The animal may be ready in three-quarters of an hour, but it may require three or four hours The degree of anesthesia is also variable, presumably due to failure of part of the drug to be absorbed

Intramuscular Method—This is the method of choice Administration is easy, anesthesia is rapidly produced, and variations in the time required for the production of anesthesia and in the depth of the anesthesia are reduced to a minimum The solution is injected deep into the gluteal muscles, one half into each side The administration is quickly and easily performed It possesses none of the disadvantages of the previous method The animal is under surgical anesthesia in from twenty to forty minutes

Intraperitoneal Method—This is a simple and fast method Satisfactory anesthesia is obtained in from fifteen to thirty minutes It has the disadvantage, however, of producing edema and hyperemia of the omentum and mesentery, and a considerable amount of fluid collects in the peritoneal cavity

Intravenous Method—This is, of course, the most rapid method Surgical anesthesia is obtained in from five to twenty minutes The injection must be made slowly to prevent the development of shock "Shock" can usually be prevented by injecting divided doses, one-third at a time, and five to ten minutes apart

The method has the advantage of being rapid If divided doses are given, however, it is hardly faster than the intramuscular method It is more difficult to administer the drug in this way as the animal must be carefully and steadily held while the saphenous vein is pierced and the injection slowly made or else it must be done under ether anesthesia

The dosage varies with the method of administration, the age, and the size of the animal The basic dose of barbitol-sodium given per os is 0.3 gm per kg The basic dose given intramuscularly or intraperitoneally is 0.27 gm per kg Intravenously the dose is 0.20 to 0.25 gm per kg Young and old dogs require smaller dosage Small animals must be given from 0.1 to 0.3 gm per kg additional whereas for large dogs the dose is decreased by from 0.1 to 0.5 gm per kg Cats require a smaller dosage, 0.25 gm per kg usually being adequate when given per os, although very small cats may require 0.3 gm per kg or even more

If the barbitolized animal shows spontaneous movements it may be quieted by injecting more barbitol or by injecting $\frac{1}{8}$ to $\frac{1}{4}$ gr of morphine sulphate After an animal has been under barbitol anesthesia for several hours it may become restless, due to reflexes set up by a distended bladder or colon In such cases the bladder or colon should be emptied

If the animal is too "deep" under the anesthetic it can frequently be made suitable for experimental work by giving from $\frac{1}{200}$ gr to $\frac{1}{100}$ gr of eserine phosphate and from $\frac{1}{2}$ gr to 1 gr caffeine

After proper anesthesia is obtained the animal should be permitted to

'sleep' (in a warm place) for a few hours. The reflexes become much more responsive.

Barbital is rather difficultly soluble. It may be neutralized by NaHCO_3 , as recommended by Tatum and Parsons, but the use of the now commercially obtainable barbital sodium is more satisfactory. Barbital sodium is soluble 1 gm in 6 cc of distilled water. The basic dose of barbital is 0.25 gm, of barbital sodium it is 0.3 gm.

Phenobarbital or *Luminal* is almost insoluble in water. It may be neutralized in $\text{N}/2$ NaOH . This sodium salt is approximately one half as soluble as barbital sodium. The dose for dogs is from 0.12 gm to 0.14 gm per 1 g, or about one half the barbital sodium dose. No advantage could be detected in its use. On the contrary it is troublesome to prepare and is markedly more toxic.

Iso amyl ethyl barbituric acid or *Amytal* is difficultly soluble in water. The sodium salt is more soluble than barbital sodium but its preparation is tedious and the solution is unstable. The manufacturers recommend 0.05 gm per kg. This dose may be increased to advantage.

Calcium ethyl isopropyl barbiturate or *Ipral* is quite difficultly soluble in water. Its use is limited therefore to per os administration. The disadvantages of this method have been pointed out above. A dosage of 0.15 gm per kg is suitable.

Thus these substances all have disadvantages not possessed by barbital sodium. Excepting those cases where survival is desired these derivatives have no advantages over barbital sodium as an anesthetic for most experimental studies such as those upon secretion and reflex action.

SUMMARY

1. The details are given for administering barbital sodium to animals for anesthesia per os intramuscularly intraperitoneally and intravenously.
2. The advantages and disadvantages of these methods are pointed out.
3. Barbital sodium is more suitable for anesthesia than barbital.
4. Luminal, Amytal and Ipral have disadvantages but no advantages over barbital sodium for anesthesia. Suitable dosages are recorded.

We wish to express our thanks to Professor Carlson for many invaluable suggestions.

REFERENCES

- ¹Tatum and Parsons. *Jour. Jour. Clin. Med.* 1922 - vol. 11.
Percy and Weaver. *Am. Jour. Physiol.*

THE DEMONSTRATION OF BACTERIOPHAGE IN OLD STOCK CULTURES*

By GORDON M. KLINE, ALBANY, N. Y.

NUMEROUS attempts have been made to demonstrate the presence of bacteriophage in so-called "normal" cultures. Bail,^{1, 2} in 1921, succeeded in isolating bacteriophage from old broth cultures of three strains of *B. dysenteriae* Flexner, and later from nine strains of *B. dysenteriae* Shiga by exhausting the broth medium with repeated inoculation and centrifugalization. Otto, Munter, and Winkler^{3, 4} and Weinberg and Aznar^{5, 6} followed this early report with similar observations of bacteriophage obtained from stock strains. Later investigations by many workers, including Seiffert,⁷ Jotten,⁸ Monteiro,⁹ Pondman,¹⁰ Gildemeister and Heizberg,¹¹ Flu,^{12, 13} Reichert,¹⁴ Burgers and Bachmann,¹⁵ and Kuttner¹⁶ and Mallmann¹⁷ in this country, failed to reveal the presence of bacteriophage in laboratory bacterial strains except in a very few exceptional instances. With regard to those cultures from which bacteriophage was isolated, many of the investigators agree with Twort¹⁸ who, in his original article describing the lytic phenomenon, reported that the lytic substance might be produced spontaneously in bacterial cultures, whereas others believe with d'Herelle¹⁹ that these cultures were contaminated with bacteriophage at the time of isolation.

The methods which were used most generally by previous workers in seeking to isolate bacteriophage from the cultures, were either a comparatively long period of incubation (ten days to six months) or a process based on the Buchner zymase extraction method—trituration with sand and subsequent extraction under pressure. The long-continued incubation, however, would be apt to obscure any bacteriophage that might have been present, for it is generally conceded that bacteria finally become resistant to the lytic action and may then destroy the bacteriophage. To quote from d'Herelle,¹⁹ p. 197, "A bacteriophage, then, becomes attenuated during the same process which leads to an acquisition of resistance by the bacterium. Even a bacteriophage of maximum virulence may be 'overcome'." Recently, Shwartzman²⁰ has shown that prolonged standing in the incubator weakens the lytic principle, even in the absence of bacteria. Since it may well be that the failure of trituration and extraction of the bacterial cell is due to the fact that the lytic substance is normally present in an inactive form, the following experimental studies were undertaken.

Experimental Work—In investigating our stock cultures, it was decided to adopt the same technic that is used in testing stool filtrates for bacteriophage. All the media employed were adjusted to P_H 7.8.

*From the Division of Laboratories and Research, New York State Department of Health. Presented in abstract before the American Association of Pathologists and Bacteriologists, April 14-15, 1927, Rochester, N. Y.

TABLE I
HISTORIES AND AGGLUTININATIVE ACTIVITY OF LYTIC CULTURES

CULTURE	HISTORY	AGGLUTINATION				
		1 200	1 1000	1 2000	1 5000	
<i>B. coli</i> communis (181)	Am Museum Natural History					
<i>B. typhosus</i> (Bender 2700)	Bender Laboratory Albany	4+	3+	3+	+	
<i>B. dysenteriae</i> Mt De crt (114 E)	N Y C Research Laboratory	4+	4+	4+	3+	
' (114 H)	Div of Lab and Research	3+	4+	4+	4+	
' (114 O)	Rockefeller Institute	4+	4+	4+	3+	
' (114 U)		4+	4+	4+	2+	
' (114 V)	College of Phys and Surg	4+	4+	3+	+	
' (114 O/1)	University of Oxford	4+	4+	4+	3+	
' (114 U/1)	Div of Lab and Research	4+	4+	4+	2+	
' (114 F)	N Y C Research Laboratory	3+	3+	2+	+	
' (114 V/1)	Pasteur Institute Paris	-	-	-	-	
' (114 D)	N Y C Research Laboratory	3+	3+	3+	2+	
<i>Staphylococcus aureus</i>	Div of Lab and Research					
<i>albus</i>						

Method—Agar slants were inoculated with the culture under investigation and incubated at 37° C for from five to seven hours. The growth was washed down with 2 c c of sterile beef-infusion broth and from 0.05 to 0.2 c c of the suspension were inoculated into 10 c c of beef infusion broth to yield a slight turbidity, 0.05 c c of this latter suspension were spread on an agar plate. After from eighteen to twenty-four hours' incubation, the tube and plate were examined for evidences of lysis. If there was any indication of lysis, the contents of the tube were filtered through a Berkefeld candle and 0.5 c c were inoculated into a fresh suspension of the bacteria and the procedure repeated.



Fig 1—*B. dysenteriae* Mt Desert No 114 D. Lytic filtrate diluted 10

By this method, lytic substance was observed in 14 of 21 cultures studied (see Table I) distributed as follows

<i>Cultures Studied</i>	<i>No. Found Lytic</i>
2 <i>B. coli</i>	1
3 <i>B. typhosus</i>	1
7 <i>B. dysenteriae</i> (Mt Desert)	7
6 <i>B. dysenteriae</i> (Shiga)	2
1 <i>B. dysenteriae</i> (Flexner)	1
2 <i>Staphylococcus</i>	2

Of the cultures found to be lytic, bacteriophage was isolated from eleven, being identified by the characteristic transmissibility in series and formation of plaques. The other three cultures, namely, the Flexner strain and the two strains of staphylococci, gave such evidences of lysis as sterile plates spread



Fig. 2—*B. dysenteriae* on nutrient agar plate.

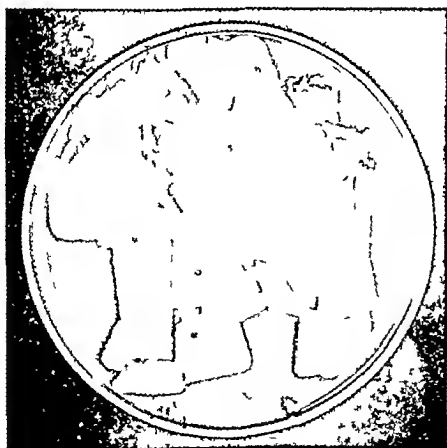


Fig. 3—*B. dysenteriae* on nutrient agar plate.

from slightly turbid suspensions, colonies becoming transparent and formation of plaques, but to date no success has followed attempts to obtain lysis in series.

The isolation of bacteriophage was not always successful on the first test, it was rather the exception to find the culture lytic at the start. Many times, neither the tubes nor the plates would show any signs of lysis. Some times, the tube would be clear and the plate show perfectly confluent growth, then again, the tube was very cloudy and the plate showed scant growth or plaques*. In many instances, duplicate tubes and plates, inoculated at the same time from the same bacterial suspension, gave these opposite extremes of growth, one tube and plate being sterile, whereas the other set showed normal growth. This variation in the lytic manifestations of the cultures is



Fig 4—*B. dysenteriae* Mt Desert No 114 U/1 Lytic filtrate diluted 10^6

very evident in the protocol included herein, giving observations of the tubes and plates inoculated with the bacteria only, over a period of a few months.

The lytic filtrates obtained from the cultures were subjected to dilution and plating in order to study the plaques so obtained. It was found that the seven strains of *B. dysenteriae* Mt Desert when plated with their homologous filtrates, gave both a large type of plaque (3 to 3.5 mm diameter), and a small type of plaque (about 1 mm diameter), the *B. typhosus* strain also developed two sizes of plaques—one about 1 mm in diameter, the other, a scarcely visible "pin-point" plaque. Only small plaques (about 1 mm diameter) were ob-

*The latter phenomenon was sometimes found to be a case of proliferation of resistant organisms in the presence of high concentration of bacteriophage as Gohs²¹ and others have observed. This was noticed very often particularly in making dilutions of the bacteriophage for estimating its strength and to obtain individual plaques. It was found that usually after from eighteen to twenty-four hours incubation the 10^{-1} and 10^{-2} dilutions were very cloudy while the others showed little growth or were perfectly sterile. By observing the tubes after from three to four hours incubation however it was found that lysis had occurred in these higher concentrations.



Fig 5—*B. dysenteriae* Mt. Desert No 114 O Lytic filtrate diluted 10



Fig 6—*B. dysenteriae* Mt. Desert No 114 O/1 Lytic filtrate diluted 10⁴. (Note the one large plaque in the upper border of the growth)

PROTOCOL 1

B dysenteriae Mt DESERT (114 E₁₀)SPONTANEOUS APPEARANCE AND DISAPPEARANCE OF LYTIC ACTIVITY
(INCUBATION PERIOD TWENTY FOUR HOURS)

DATE	TUBE	PLATE	DATE	TUBE	PLATE
1927			1927		
1/20	4+	4 transparent colonies	2/14	4+	Scattered growth
	-	Confluent growth	2/15	4+	Confluent growth
1/24	-	Confluent growth	2/18	3+	Confluent growth
1/25	4+	Many plaques	3/1	-	Confluent growth
	-	Confluent growth	3/9	3+	45 large plaques
	4+	Sterile			3 small plaques
1/26	-	Confluent growth	3/10	+	7 large plaques
2/1	-	Confluent growth	3/12	-	Confluent growth
2/2	4+	Many plaques	3/14	-	2 large plaques
	-	Confluent growth		-	Confluent growth
2/3	-	Confluent growth	3/15	-	70 small plaques
2/10	4+	4 large plaques	3/18	4+	4 large plaques
					100 small plaques
					12 large plaques

tained from the *B coli* and the two Shiga strains under the same conditions (Figs 1 to 12) These results seemed to indicate two distinct lytic mechanisms operating in some bacteria In order to prove the independence of



Fig 7—*B coli communis* No 186 Lytic filtrate diluted 10^4

the two mechanisms, the large and small plaques from the Mt Desert strains were fished and replated separately with the homologous strain It was observed that the large plaques gave rise to only large plaques and the small plaques to small ones* Moreover, the filtrates containing lysin developing

*This is true only when the strain itself is not at the lytic threshold as will be shown later

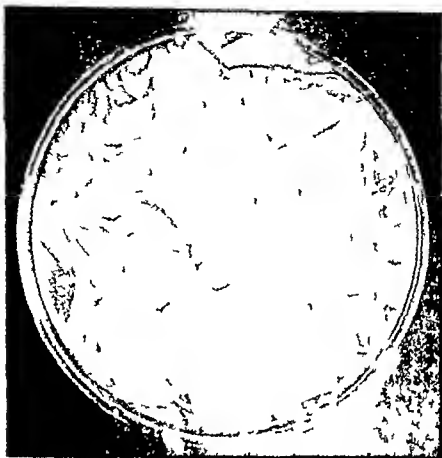


Fig 8—*B. typhosus* No 201 Lytic filtrate diluted 10



Fig 9—*B. dysenteriae* Shiga No 114 F Lytic filtrate diluted 10

small plaques were active against some species on which the lysin, identified by the large plaques, had no effect. The separate entity of each of the two lytic mechanisms operating spontaneously in the Mt Desert strains studied is, therefore, established. Furthermore, the identity of the large plaques and of the small plaques obtained from each of the seven Mt Desert strains characterizes the lytic manifestations as inherited processes rather than due to chance contamination with foreign parasites.

One of the Mt Desert strains used in this study, had, in the course of another experiment, been fished every six months from a single colony during the five-year period from 1920 to 1925, and the fishing used to continue the strain. D'Herelle has found that this procedure with cultures either artificially or naturally containing bacteriophage will eliminate the bacterio-



Fig. 10—*B. dysenteriae* Shiga No. 114 V/1. Lytic filtrate diluted 10^{-4} .

phage from the strain except in very rare cases. It was thought possible, therefore, that the above culture would either be free of lytic substance entirely or that one of the bacteriophagic types (represented by either the large plaque or the small plaque) would have been removed. Examination of this culture, however, designated as 114 E₁₀ showed that the ten colony isolations had eliminated neither the lytic properties of the culture as a whole nor either one of the lytic mechanisms active in the strain before colony isolation.

A study of the agglutinability of the cultures from which bacteriophage was isolated gave results contrary to the view commonly expressed in the literature with regard to the agglutination of lytic cultures (or cultures "contaminated" with bacteriophage). D'Herelle¹⁹ states, on page 236, that, "the bacteria of contaminated strains are but slightly or not at all agglutinable by a specific antiserum," and recently, Hadley²² has confirmed this

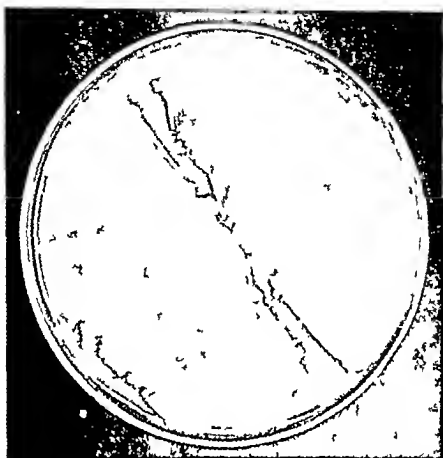


Fig 11 — *Staphylococcus aureus* No 56 B



Fig 1 — *Staphylococcus aureus* No 56 A

statement The agglutination of all our cultures, however, was normal (see Table I) with only one exception The correlation of agglutinability with bacteriophagic phenomena may perhaps have been overemphasized Bacteria freshly isolated from human infections are often practically magglutinable Metchinkoff and Bordet²³ found that bacterial strains showing normal agglutination became magglutinable by remaining with leucocytes, Dawson⁴ has reported considerable variation in the agglutinability of identical strains of bacteria grown in different media Many similar instances of the effect of controlled environmental changes on the agglutination of bacteria have been demonstrated In view of these observations, magglutinability is not necessarily an accurate criterion of the presence of bacteriophage in bacterial strains

DISCUSSION

It has been d'Herelle's contention that stock cultures in which bacteriophage has been found were contaminated by the ultravirus when originally isolated The accumulation of observations, however, seems to indicate that the lytic state may occur spontaneously at a certain stage in the metabolism of the organisms Bordet²⁵ and Insbonne and Carrère²⁶ found that *B. coli* strains became lytic spontaneously and as quickly became "normal" again Hadley²⁷ found that *B. pyocyaneus* suddenly became lytic after having grown normally through many generations Bagger²⁸ in a recent study of the enterococcus mentions that one of his strains "which previously had many times been inoculated in the same manner" suddenly gave rise to extensive bacteriophagous activity The observations on the cultures recorded in this paper reveal not only spontaneous appearance and disappearance of bacteriophage action in bacterial cultures, but also an extreme variation in the lytic activity of bacteria from the same agar slant inoculated in duplicate into broth medium and immediately spread on agar In the latter instance, it was noted that one agar surface was completely sterile after twenty-four hours, while the other showed perfectly confluent growth It is difficult to conceive that such a phenomenon depends on the chance circumstance that one agar surface had been spread with bacteria for the most part contaminated with a foreign parasite, whereas the bacteria spread on the other agar surface had been entirely free of the parasite It is more likely that the phenomenon is related to other spontaneous dissociations, now widely recognized, occurring constantly among the various bacterial species

The apparent hereditary character of the bacteriophage races obtained spontaneously from the lytic cultures is very striking It is generally conceded that, although the size of the plaques may be made to vary by changing the environmental conditions, nevertheless, under the same conditions, a plaque, when fished and plated with fresh culture, will develop many more of the same size This has been found to be true for both the large and the small Mt Desert plaques It must be thought, therefore, that a large plaque represents a definite lytic mechanism (B) as compared to that mechanism (b) developing small plaques Since plaques of two different sizes appeared on the plates of the lytic Mt Desert strains and of the lytic *B. typhosus*

strains, we have experimental evidence of the presence of at least two distinct lytic mechanisms in some bacterial cells. This presence of more than one bacteriophage race in a single strain readily explains the phenomenon observed by Seiffert²⁰ that the development of resistance to one lytic agent might be accompanied by a newly acquired sensitiveness to another. He, and later Burnet²⁰ reasoned there must be more than one lytic ferment present, and this I have found to be the case.

Hadley,³¹ in 1924, found that a lytic filtrate of the "small" strain only, acting on Shiga gave only small plaques whereas a lytic filtrate of the "large" strain gave both large and small plaques. He suggests that the exciting cause is susceptible of independent variation and compares the phenomenon with his nonlytic and lytic pyocyanens colonies the first giving rise to nonlytic colonies only, the second to both lytic and nonlytic colonies. In my experiments in which the large and small plaques of the Mt Desert culture were fished and replated separately, it was usually possible to obtain each one to the exclusion of the other but occasionally one or more of the series would show a mixture of plaques. Inasmuch as the control plates also gave plaques, separately or mixed spasmodically the occasional appearance of both types on a plate to which only one type plaque was fished is evidence merely that the culture itself was approaching the lytic threshold when it would spontaneously produce bacteriophage and that the stimulus applied to one lytic mechanism by the filtrate added was sufficient to discharge another also. Only small plaques were found in the two Shiga cultures giving bacteriophage spontaneously, this would seem to indicate that the mechanism (b) in the Shiga bacillus is easily set off in Hadley's case by the mere operation of another mechanism (B).

SUMMARY

Previous investigations of old stock cultures have demonstrated bacteriophage in only a small percentage of cases possibly due to the methods employed. When the technique used in examining stool filtrates for bacteriophage was applied to stock cultures lytic substance was observed in 14 of 21 cultures studied. Typical bacteriophage as identified by transmissibility in series and formation of plaques was obtained from 11 of the 14 lytic cultures namely, 1 *B. coli*, 1 *B. typhosus*, 2 *B. dysenteriae* Shiga and 7 *B. dysenteriae* Mt Desert strains. Tubes and plates inoculated with the bacteria only, over a period of a few months varied from complete lysis to "normal" growth. In some instances duplicate tubes and plates inoculated at the same time from the same bacterial suspensions gave these opposite extremes. This spontaneous appearance and disappearance of bacteriophagic action in these cultures associates the lytic phenomenon with other spontaneous microbial variations. The 7 *B. dysenteriae* Mt Desert strains gave both large plaques (3 to 3.5 mm diameter) and small plaques (about 1 mm diameter) indicating the presence of at least two distinct lytic mechanisms characterized as inherited processes rather than as chance contaminations with foreign parasites. One of the Mt Desert strains was fished from isolated colonies ten times over a period of five years but this procedure failed to eliminate either the lytic

property of the culture as a whole, on either one of the lytic mechanisms active in the strain before colony isolation. Although d'Hérelle states that "the bacteria of contaminated strains are but slightly or not at all agglutinable by a specific antiserum," the agglutinability of the cultures containing bacteriophage was normal with only one exception.

REFERENCES

- ¹Bail, O. Bakteriophage Wirkungen gegen Flexner und Kolibakterien, Wien klin. Wchnschr, 1921, *lxiv*, 148
- ²Bail, O. Über Shiga Bakteriophagen, Wien klin. Wchnschr, 1921, *lxiv*, 555
- ³Otto, R., and Munter, H. Zum d'Hérélleschen Phänomen, Deutsche med. Wchnschr, 1921, *xlvi*, 1579
- ⁴Otto, R., and Winkler, W. F. Über die Natur des d'Hérélle'schen Bakteriophagen, Deutsche med. Wchnschr, 1922, *xlvi*, 383
- ⁵Weinberg, M., and Aznar, P. Antibacteriolysines et le phénomène de d'Hérélle, C. r. Soc. de Biol, 1922, *lxxvi*, 833
- ⁶Weinberg, M., and Aznar, P. Quelques faits nouveaux sur les antibacteriolysines, C. r. Soc. de Biol, 1922, *lxxvii*, 136
- ⁷Seiffert, W. Über das d'Hérélleschen Phänomen, Soziale Hyg. u. Mikrobiol, Sept. 21, 1922, Cited by d'Hérélle¹⁰
- ⁸Jotten, K. W. Über das sogenannte d'Héréllesche Phänomen, Klin. Wchnschr, 1922, *i*, 2181
- ⁹Monteiro, J. L. Sobre o phenomeno de d'Hérélle. Presença do principio lytico nas culturas, em meio solido, do Bacillo anthracis, B. pestis e B. dys Shiga Kruse, Brazil Med, 1922, *i*, 297
- ¹⁰Pondman, A. Proeven tot het verkrijgen van het verschijnsel van d'Hérélle uit rein culturen, Leiden C. de Boer jr., 1923, Cited by d'Hérélle¹⁰
- ¹¹Gildemeister, E., and Herzberg, K. Über das d'Héréllesche Phänomen. III. Mitteilung, Centralbl. f. Bakteriol, 1924, *xc*, 12
- ¹²Flu, P. C. Die Natur des Bakteriophagen und die Bildung von Bakteriophagen in alten Bouillonkulturen pathogener Mikroorganismen, Centralbl. f. Bakteriol, 1923, *xc*, 362
- ¹³Flu, P. C. Over Cholera-bacteriophagen, Geneesk. Tijdschr. v. Vergeehk, 1924, *v*, 196, Cited by d'Hérélle¹⁰
- ¹⁴Reichert, F. Untersuchungen über das d'Héréllesche Phänomen, Centralbl. f. Bakteriol, 1924, *xc*, 235
- ¹⁵Burgers, and Bachmann, W. Bakteriophagen studien. I. Versuche mit grampositiven Mikroorganismen, Ztschr. f. Hyg. u. Infektionskrankh., 1924, *ci*, 350
- ¹⁶Kuttner, A. G. Bacteriophage Phenomena, Jour. Bact., 1923, *viii*, 49
- ¹⁷Mallmann, W. L. Old Stock Cultures As a Source of Bacteriophage, Jour. Bact., 1923, *v*, 59
- ¹⁸Twort, F. W. An Investigation on the Nature of Ultra Microscopic Viruses, Lancet, 1915, *clxxxix*, 1241
- ¹⁹d'Hérélle, F. The Bacteriophage and Its Behavior, Baltimore, Williams and Wilkins, 1926
- ²⁰Shwartzman, G. Observations on Lytic Principle of Weak Potency, Proc. Soc. Exper. Biol. Med., 1924, *25*, *xxii*, 433
- ²¹Gohs, W. Die lytische Wirkung des Shiga-bakteriophagen bei verschiedenen Konzentrationen, Wien klin. Wchnschr, 1925, *lxviii*, 481
- ²²Hadley, P. Microbic Dissociation, the Instability of Bacterial Species with Special Reference to Active Dissociation and Transmissible Autolysis, Jour. Infect. Dis., 1927, *xl*, 1
- ²³Bordet, J. Studies in Immunity, New York, John Wiley and Sons, 1909
- ²⁴Dawson, A. I. Bacterial Variations Induced by Changes in the Composition of Culture Media, Jour. Bact., 1919, *xv*, 133
- ²⁵Bordet, J. Apparition spontanée du pouvoir lysogène dans les cultures pures, C. r. Soc. de Biol, 1924, *xc*, 96
- ²⁶Lisbonne, M., and Carrere, L. Sur l'apparition spontanée du pouvoir lysogène dans les cultures pures, C. r. Soc. de Biol, 1924, *xc*, 265
- ²⁷Hadley, P. Transmissible Lysis of Bacillus Pyocyaneus, Jour. Infect. Dis., 1924, *xxxiv*, 260
- ²⁸Bagger, S. V. The Enterococcus, Jour. Path. Bact., 1926, *xxix*, 225
- ²⁹Seiffert, W. Der Charakter des d'Hérélleschen Phänomens, Ztschr. f. Immunitätsforsch. u. exper. Therap., 1923, *xxxviii*, 292
- ³⁰Burnet, F. M. The Nature of the Acquired Resistance to Bacteriophage Action, Jour. Path. Bact., 1925, *lxviii*, 407
- ³¹Hadley, P. The Variation in Size of Lytic Areas and Its Significance, Jour. Bact., 1924, *ix*, 397

A COMPARISON OF 10,000 WASSERMANN AND KAHN TESTS RUN IN PARALLEL*

BY LAWRENCE D. THOMPSON, M.D. AND ELSA EBEL, ST. LOUIS, MO

SINCE the introduction of a flocculation test for the diagnosis of syphilis by Kahn¹ several years ago several articles have appeared in the literature comparing the advantages or disadvantages of this test with those of the Wassermann reaction. A bibliography of the most important contributions is appended to this report.

Although many articles have been published on this subject we feel justified in presenting the results of this series of tests because we believe that our figures portray a comparison of the two tests more accurately than the majority of the previous reports. Many have reported mere percentages of the agreement and disagreement between the two tests. Such figures are based on the total number of tests run and do not take into consideration the presence or absence of syphilis in the patients whose sera are tested. These reports have shown an approximate agreement between the two tests in 88 to 96 per cent of the tests run. In the remaining 4 to 12 per cent about one half, or 2 to 6 per cent, of the tests have shown a positive Wassermann and a negative Kahn and a like number have shown a negative Wassermann and a positive Kahn. There have also been a number of reports in which a careful clinical analysis has been made. Such reports naturally give more accurate information regarding the two tests in the presence of syphilis but in these reports also the percentages are based on the total number of tests run.

Houghton, Hunter and Cayigas,² in a comprehensive report of a large series of tests have presented the most convincing arguments for the adoption of the Kahn test in place of the Wassermann test. They conclude that the Kahn test is more sensitive than the Wassermann test in all stages of syphilis but more particularly so in cases of primary syphilis and in treated cases. In view of the many technical advantages of the Kahn test and its equal reliability with the Wassermann test they recommend the adoption of the Kahn test in place of the Wassermann test.

The other side of the question has been presented quite adequately by Kilduffe³ who points out a number of real dangers and disadvantages of the Kahn test. He mentions the relative difficulty in securing a satisfactory Kahn antigen and the necessity of frequent titration of the antigen. He also emphasizes the importance of having the Kahn test conducted only by a thoroughly trained and competent technician.

TECHNIC

The following Wassermann technique is used in this laboratory. Two antigens are used, one being a simple alcoholic beef heart extract antigen, and

From the Department of Internal Medicine, Washington University School of Medicine and Barnes Hospital.

Received for publication April 30, 1917.

the other being the same antigen to which cholesterol (0.6 per cent) has been added. A 5 per cent suspension of sheep cells is used and the hemolytic system is titrated before each test. The amount of serum used is 0.1 cc. The tests with the cholesterolized antigen undergo a one-hour water-bath fixation while the tests with the alcoholic antigen are subjected to a four-hour ice box fixation. We believe that such technique makes the two antigens about equally sensitive. The Kahn test is run strictly in accordance with the instructions given by Kahn.¹ The same cholesterolized antigen is used for both the Wassermann and Kahn tests and the Kahn tests are read after a period of fifteen to eighteen hours' incubation at 37.5° C.

TABLE I*

A COMPARISON OF 10,000 WASSERMANN AND KAHN TESTS RUN IN PARALLEL. THE PERCENTAGES BASED ON THE TOTAL NUMBER OF TESTS RUN

WASSERMAN AND KAHN AGREE THROUGHOUT				
	++++	6.16%		
	+++	0.16		
	++	0.17		
	+	0.13	6.62	
	-		69.60	76.22
WASSERMANN AND KAHN AGREE QUALITATIVELY BUT DIFFER QUANTITATIVELY				
<i>Kahn More Sensitive—</i>				
WC equals K both stronger than WA		9.33		
WA equals K both stronger than WC		0.19		
K stronger than either antigen of W		6.11	15.63	
<i>Wassermann More Sensitive—</i>				
WA equals WC both stronger than K		0.33		
WC stronger than either WA or K		1.05		
WA stronger than either WC or K		0.24	1.62	17.25
<i>Wassermann and Kahn Show Complete Discrepancy—</i>				
K pos W neg		3.33		
W pos K neg		3.20		6.53
				100.00

*This table is similar to the many tables already in the literature and gives the percentages of agreement and disagreement between the two tests based on the total number of tests in the series. We found a complete quantitative agreement in 76.22 per cent of the tests. In 17.25 per cent of the tests there was a qualitative agreement but a quantitative difference. Of these differing quantitatively 15.61 per cent were in favor of the Kahn test and only 1.62 per cent in favor of the Wassermann test. Combining the two groups we found a qualitative agreement in 93.47 per cent of the tests run. In 6.53 per cent of the tests there was a complete discrepancy between the Wassermann and Kahn results. Of these 3.33 per cent gave a negative Wassermann and a positive Kahn and 3.2 per cent gave a positive Wassermann and a negative Kahn.

TABLE II†

CLINICAL ANALYSIS OF TESTS SHOWING AN ABSOLUTE DISCREPANCY 653 CASES

	UNDETERMINED	SYPHILITIC	NONSYPHILITIC	TOTAL
K pos W neg	9	261	63	333
W pos K neg	2	199	119	320
	11	460	182	653

†This table is an attempt to show the results of a clinical analysis of those cases which gave an absolute discrepancy between the Wassermann and Kahn Tests. We have designated as syphilitic those cases which either presented clinical syphilis or gave a definite history of having had a chancre or a previously frankly positive serology which had been altered by treatment. The cases designated as nonsyphilitic neither presented the clinical picture of syphilis nor gave a history suggesting syphilis and in many of these cases subsequent tests revealed a negative serology. We admit that such a division is not free from the possibility of error but it does give as accurate information regarding the presence of syphilis as is possible to secure. Of the 653 cases coming under this group there were eleven in which the clinical analysis was impossible so we have designated these cases as undetermined.

DISCUSSION

The test which gives the more accurate information regarding the presence or absence of syphilis in the patient whose serum is tested is the test which will be adopted eventually. Figures based on the total number of tests run will vary with the number of negative tests in the series. For this reason percentages, to be of value must be based on the reaction of the tests in a series of sera from syphilitic patients. Furthermore a test which is so sensitive that it gives a reaction in the absence of syphilis is almost as objectionable as one which fails to reveal syphilis when present. It is our opinion that such procedures as a prolonged ice box fixation may very well increase the sensitivity of the Wassermann reaction beyond the point of specificity. This danger is not so apparent with the Kahn test. The ability of the average practitioner to interpret correctly the results of the Wassermann test in

TABLE III
COMPARISON OF THE WASSERMANN AND KAHN TESTS IN 2347 SYPHILITIC SERA

Total sera 2347	K Neg in 199	Failure of Kahn = 6.9 per cent
Total sera 2347	W Neg in 261	Failure of Wass = 9.1 per cent

COMPARISON OF WASSERMANN AND KAHN TESTS WITH REGARD TO 'FALSE POSITIVES'			
Percentages based on number of known false reactions (Table II) and total number of positive reactions			
Pos Kahn reactions 2720	False pos 63	Falsely pos 2.3 per cent	
Pos Wass reactions 2767	False pos 119	Falsely pos 4.4 per cent	

*This table shows a comparison of the two tests with sera from syphilitic patients. We are assuming that the 66⁹ sera (Table I) where we found quantitative agreement with positive reactions are all from syphilitic patients. We also assume that the 1, 5 sera (Table I) which showed only a qualitative agreement but again all positive reactions are from syphilitic patients. We know from analysis that the 460 sera (Table II) of the complete discrepancy group are from syphilitic patients. The sum of these groups make a total of 847 syphilitic sera.

Such a comparison demonstrates without a doubt that neither test is infallible and that the error with either test is actually much greater than other reports have indicated. The Kahn test however is definitely more dependable where there is the question of diagnosis. The table also indicates that each test will give an appreciable number of false positive reactions but here also the Kahn test appears to be definitely more reliable.

sera which gives only a partial reaction is variable. The laboratory man who conducts the tests is able, no doubt to interpret his results according to the technic which he has employed, but it must be remembered that the general practitioner may attach an entirely different significance to the reaction. A glance through the literature will readily reveal the fact that some laboratory men consider a one or two plus reaction, with only one antigen, in the Wassermann test as a positive and significant test while other men consider such a reaction as a negative test. A partial reaction with the Kahn test is more uniformly interpreted as being significant.

As has been pointed out by Kilduffe² the Kahn test is surrounded by far too many pitfalls to be safely employed as an office procedure by the general practitioner. A source of error which has not been emphasized in the literature is the comparative thermolability of the Kahn reaction. This fact makes the inactivation of the serum a procedure which must be conducted most carefully and at the lowest temperature possible.

It will be noted that in the 3.2 per cent of the tests (Table I) where the Kahn apparently failed, syphilis was present in only 1.99 per cent and two of

the cases were "undetermined" This indicated that the Kahn actually failed in less than 2 per cent of the tests run Reasoning in a similar manner, in the 3.33 per cent where the Wassermann apparently failed, syphilis was present in 2.6 per cent with nine cases "undetermined" So the Wassermann failed in at least 2.6 per cent of the total number of tests run

TABLE IV

ANALYSIS OF ABSOLUTE DISCREPANCIES—653 CASES = 6.53 PER CENT OF TESTS RUN

	K	W C	W A.	NUMBER OF CASES	TOTAL	KNOW FALSE REACTIONS	TOTAL
<i>K pos W neg</i>	++++	-	-	8		1	
	+++	-	-	30		4	
	++	-	-	235		43	
	+	-	-	60	333	15	63
<i>W pos K neg</i>	-	++++	++++	10		4	
<i>W C equals W A</i>	-	+++	+++	5		1	
	-	++	++	7		2	
	-	+	+	28	50	10	17
<i>W C stronger than W A</i>	-	++++	-	6		1	
	-	+++	++	3		0	
	-	+++	+	7		1	
	-	+++	-	8		2	
	-	++	+	14		3	
	-	++	-	52		18	
	-	+	-	136	226	63	88
<i>W A stronger than W C</i>	-	++	++++	6		3	
	-	+	++++	7		3	
	-	-	++++	8		3	
	-	++	+++	3		0	
	-	+	+++	4		0	
	-	-	+++	5		1	
	-	+	++	8		3	
	-	-	++	2		1	
	-	-	+	1	44	0	14
						653	119
							132

Key to Tables

K = Kahn

W = Wassermann

W A = Wassermann alcoholic

W C = Wassermann cholesterol

Neg = Negative

Pos = Positive

This table gives a more detailed picture of the group of cases represented by Table II. In those sera which showed a complete discrepancy the degree of reaction between the two tests is shown. The degree of the false positive tests is also shown. It is significant to note that the complete discrepancies between the two tests occurred for the most part in sera reacting weakly with either one or the other test. The false positive reactions with both tests also occurred for the most part in weakly reacting sera.

CONCLUSIONS

- 1 The Kahn test fails to detect syphilis in 6.9 per cent of cases
- 2 The Wassermann test fails to detect syphilis in 9.1 per cent of cases
- 3 In the diagnosis of syphilis the Wassermann and Kahn tests should preferably be run in parallel
- 4 The Kahn test is more specific than the Wassermann test (At least when compared with the Wassermann technic as used in this laboratory, and that technic is sensitive enough to give reactions beyond the point of specificity)

REFERENCES

- ¹Kahn, R L The Kahn Precipitation Test for Syphilis, Am Jour Pub Health 1924, xiv 498
Kahn, R. L Serum Diagnosis of Syphilis by Precipitation Governing Principles Procedure and Clinical Application of the Kahn Precipitation Test Baltimore, Williams & Wilkins Co., 1925
- ²Anderson, C M Value of Parallel Kolmer and Kahn Tests as Standardized Laboratory procedure in Serum Diagnosis of Syphilis Canadian Med Assn Jour, 1926, xvi 1211
Becker S W Comparison of the Kahn Precipitation Test and the Kolmer Complement Fixation Test, with Consideration of the Difficulties in the Diagnosis of So Called Latent Syphilis, Am Jour Syph 1927 xi 131
Duenling W W Clinical Observations on the Kahn Test Jour Ind Med Assn, July, 1926 xix, 272
Giordana, A S Kahn Precipitation Test is Compared with Kolmer Complement Fixation Test, JOUR LAB AND CLIN MED Feb 1926 xi 439
Greenbaum S S Error of Basing Serum Diagnosis of Syphilis on the Kahn Reaction Alone, Jour Am Med Assn 1926 lxxxvi 1273
Hopkins, J G and Brunet W M The Kahn Precipitation Test for Syphilis, Jour Am Med. Assn., 1927, lxxxviii 311
Houghton, J E Hunter, O B, and Cajigas T The Kahn Test Jour Am Med. Assn., 1926, lxxxvii 1668
Kelly R L Clinical Study of Kahn Precipitation Test and Kolmer Complement Fixation Test, Arch Derm Syph Nov 1925 xi 720 JOUR LAB CLIN MED Feb, 1926, xi 437
Kilduffe Roht A Wassermann vs Flocculation Tests in the Serological Study of Syphilis Suitability of Kahn Test as an Office Procedure Arch Path and Lab Med, 1926 ii, 866
Lederer A Comparative Value of Wassermann and Kahn Tests U S Vet Bur Med Bull, May, 1926 ii 456
Lutterer W Comparison of the Wassermann Test with the Kahn Test in 17,000 Serums Jour Tenn. St Med Assn Sept 1926 xix
McGlumphy, C B Flocculation Test for Syphilis Arch Path Lab Med 1926 ii, 769
Owen R G, and Cope H E Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test JOUR LAB CLIN MED Feb 1926 xi 432
Redfield K T The Kahn Test as an Aid in the Diagnosis of Syphilis Am Jour Syph, 1926, ix, 354

LABORATORY METHODS

THE DETERMINATION OF FIBRIN IN BLOOD PLASMA*

By JOSEPH CHANDLER, PH D., BOSTON, MASS

IN the fall of 1925 I undertook a series of chemical studies of the blood during pregnancy. Among the constituents of which the concentration was to be determined, was fibrin. While the concentration of fibrin in blood has been determined for a long time, it is only within a comparatively few years that methods which offer a reasonable degree of speed, accuracy and economy of material have been available. Some of the earlier methods have been described by Meek,¹ who as late as 1912 determined fibrin by whipping a measured quantity of whole blood, grinding, washing with water and sodium chloride solution until the wash liquor was biuret free, filtering off the fibrin, and, after washing with alcohol and ether, drying and weighing directly.

Within the past five years several excellent methods have been published. In most cases oxalate or citrate plasma has been used, which, after dilution with sodium chloride solution, is recalcified, allowed to clot, the fibrin removed, washed and either dried and weighed or the nitrogen determined by some modification of the Kjeldahl method. A review of twelve typical methods is given by Stallinge.² Cullen and Van Slyke,³ and Howe⁴ use the Kjeldahl method, while Giam,⁵ and Foster and Whipple⁶ prefer the gravimetric method. The refractometric method has been applied to the determination of fibrin in blood by Winteritz,⁷ Leendertz and Gromelski,⁸ and Leendertz.⁹ An earlier method in which the smallest volume of magnesium sulphate plasma which will coagulate when mixed with a definite volume of fresh serum, is that proposed by Wohlgemuth.¹⁰

Wu¹¹ has recently proposed a very ingenious procedure. One c.c. of oxalate plasma is diluted with 28 c.c. of 0.8 per cent sodium chloride solution, recalcified with 1 c.c. of 2.5 per cent calcium chloride solution and the mixture allowed to clot for twenty minutes, the clot is loosened by gentle shaking and poured on a dry filter. The fibrin is removed by winding on a fine glass rod, dried with filter paper and placed in a 15 c.c. graduated centrifuge tube. Four c.c. of 1 per cent sodium hydroxide solution are added and the mixture heated in a boiling water bath until the fibrin is disintegrated. When cool, 10 c.c. of water are added and mixed thoroughly and the mixture centrifuged. The clear supernatant liquid is poured into a 25 c.c. volumetric flask, 1 c.c. of 5 per cent sulphuric acid, 0.5 c.c. phenol reagent and 3 c.c. of 20 per cent sodium carbonate solution are added, and after making up to volume and letting stand for fifteen minutes the color is compared with that of a standard tyrosine solution.

*From the Evans Memorial and Boston University School of Medicine
Received for publication Feb. 19, 1927.

Wu's method seemed to offer the three advantages of accuracy, rapidity and economy of material, and was adopted tentatively for the investigation. The results obtained, however, were not entirely satisfactory. There was a strong tendency for a white precipitate to form during the fifteen minutes' interval of standing prior to the color comparison. The color also seemed to vary somewhat with the time of heating with the sodium hydroxide, and this difficulty could not be avoided easily as the heating must be continued until the fibrin is completely disintegrated.

Finally this method was modified with satisfactory results. The method of isolation of the fibrin suggested by Wu was retained, but for his colorimetric method, the determination of the nitrogen in the precipitated fibrin by a modification of the Folin microkjeldahl method was substituted. Results from duplicate determinations on the same plasma sample gave good agreement as is shown in Table I.

TABLE I
AGREEMENT OF DUPLICATE DETERMINATIONS

NO	NAME	PER CENT	PLASMA		FIBRIN	CLASSIFICATION
			I	II	Mean	
1	R. M.	0.371%	0.271%	0.271%	0.240%	Normal
2	A. G.	0.267%	0.267%	0.267%	0.267%	Normal
3	E. T.	0.399%	0.377%	0.384%	0.384%	Normal
4	L. H.	0.254%	0.243%	0.249%	0.249%	Normal
5	E. R.	0.258%	0.253%	0.255%	0.255%	Normal
6	E. R.	0.234%	0.217%	0.226%	0.226%	Normal
7	F. K.	0.313%	0.313%	0.313%	0.313%	Normal
8	L. S.	0.237%	0.243%	0.240%	0.240%	Normal
9	L. S.	0.128%	0.122%	0.125%	0.125%	Menstruation
10	L. S.	0.233%	0.241%	0.237%	0.237%	Normal
11	L. S.	0.211%	0.206%	0.208%	0.208%	Menstruation
12	E. W.	0.274%	0.277%	0.276%	0.276%	Normal
13	E. W.	0.233%	0.230%	0.234%	0.234%	Menstruation
14	R. P.	0.308%	0.300%	0.304%	0.304%	Menstruation
15	G. C.	0.377%	0.52%	0.61%	0.61%	Pregnancy normal
16	I. R.	0.389%	0.404%	0.406%	0.406%	Pregnancy normal
17	M. R.	0.408%	0.451%	0.459%	0.459%	Pregnancy normal
18	E. C.	0.490%	0.490%	0.490%	0.490%	Pregnancy normal
19	B. B.	0.430%	0.438%	0.437%	0.437%	Pregnancy normal
20	R. H.	0.346%	0.342%	0.344%	0.344%	Pregnancy normal
21	A. DeC.	0.383%	0.398%	0.398%	0.398%	Pregnancy normal
22	E. S.	0.255%	0.243%	0.249%	0.249%	Pregnancy normal
23	A. D.	0.293%	0.400%	0.391%	0.391%	Pregnancy normal second month
24	A. D.	0.181%	0.188%	0.187%	0.187%	Pregnancy normal fourth month
25	A. D.	0.411%	0.406%	0.409%	0.409%	Pregnancy normal fifth month
26	A. D.*	0.411%	0.401%	0.409%	0.409%	Pregnancy normal seventh month
27	R. G.	0.434%	0.474%	0.446%	0.446%	Pregnancy hypertension
28	L. E.	0.447%	0.494%	0.471%	0.471%	Pregnancy hypertension
29	F. D.	0.472%	0.463%	0.470%	0.470%	Pregnancy toxemia
30	C. R.	0.413%	0.421%	0.416%	0.416%	Pregnancy thyrotoxicosis
31	L. A.	0.875%	0.985%	0.932%	0.932%	Pregnancy post cesarean phlebitis
32	H. W.	0.494%	0.461%	0.478%	0.478%	Pregnancy, placenta previa
33	E. G.	0.502%	0.486%	0.494%	0.494%	Pregnancy placenta previa
34	F. G.	0.420%	0.411%	0.417%	0.417%	Pregnancy fourth month psychosis
35	N. T.	0.632%	0.619%	0.625%	0.625%	Pregnancy eclampsia
36	C. P.	0.644%	0.644%	0.644%	0.644%	Pregnancy eclampsia
37	M. P.	0.632%	0.613%	0.623%	0.623%	Pregnancy gastrointestinal
38	Mr. S.	0.137%	0.140%	0.139%	0.139%	Banti's disease
39	Mr. S.	0.131%	0.137%	0.134%	0.134%	Banti's disease

*Third Value 0.469%

†Third Value 0.415%

Experiment showed that, with normal bloods at least, clotting was complete in thirty minutes as evidenced in Table II. This is also stated to be the case by Foster and Whipple.⁶

TABLE II
EFFECT OF VARYING CLOTTING TIME VALUES IN PER CENT PLASMA FIBRIN
TIME IN MINUTES

NAME		30	60	120	180	240	MINUTES
F	1	0.314	0.300	0.308	0.274	0.300	
	2	0.318	0.290	0.305	0.300		
	Mean	0.316	0.295	0.307	0.287	0.300	
C	1	0.266	0.267	0.264	0.266	0.266	
	2	0.265	0.265	0.265	0.257	0.263	
	Mean	0.266	0.266	0.265	0.262	0.264	
W	1	0.283	0.262	0.268	0.276	0.267	
	2	0.273	0.261	0.265	0.286		
	Mean	0.278	0.262	0.267	0.281	0.267	

In the case of only one plasma sample has clotting failed to take place, and in this case the mixture remained entirely liquid after several hours standing and the addition of calcium chloride a second time.

If it were the universal custom to use the Kjeldahl method for the determination of fibrin, it would seem logical to express results as fibrin nitrogen rather than as fibrin. But in view of the large number of published results obtained by gravimetric methods and the continued use of this procedure, it has seemed best to tabulate results as fibrin. The fibrin values have been obtained by multiplying the nitrogen values by 6.25. While Hammarsten gives the percentage of nitrogen in horse fibrin as 16.91 per cent, which would give a factor of 5.91, in the absence of any recent study of the nitrogen percentage of human fibrin, it has seemed best to employ the conventional factor 6.25.

It is the custom of many workers to calculate the fibrin concentration on the basis of whole blood, the corpuscle-plasma ratio being determined by the hematocrit method. In my opinion, such calculations are of questionable accuracy. While in one paper⁶ it is stated that it is possible to express all plasma from between the corpuscles by centrifuging at 3000 R P M for thirty minutes, it would seem that this statement is open to question. In my opinion it is much better to express the fibrin concentration in terms of plasma. All results in this paper, therefore, are expressed as per cent plasma fibrin, that is, grams of fibrin (fibrin nitrogen \times 6.25) per 100 cc of plasma.

DETAILS OF PROCEDURE

Blood is drawn from the vein by means of a syringe and introduced at once into a bottle previously prepared with the required quantity of solid lithium or potassium oxalate by measuring into it that volume of a standard solution of the oxalate required to furnish 1 mg of the lithium salt or 2 mg of the potassium salt per cc of blood. Water is expelled by drying in an oven. After addition of the blood, a rubber stopper is inserted into the bottle and the contents shaken thoroughly but not violently, as violent shaking

sometimes causes laking, this tends to contaminate the fibrin with corpuscle protein and give results above the true value. The blood is now centrifuged for twenty to thirty minutes at about 2000 R P M and the plasma carefully drawn off with a medicine dropper with a fine tip into another centrifuge tube. It is much more satisfactory to remove the plasma in this manner than to attempt to measure with a pipette directly from the centrifuge tube containing both plasma and corpuscles.

One c.c. of clear plasma is measured with a calibrated Ostwald Folin pipette into a 25 x 200 mm test tube. 29 c.c. of 0.8 per cent NaCl and 1 c.c. of 2.5 per cent CaCl_2 are added and the contents mixed and allowed to stand for at least thirty minutes. At the end of that time a rather firm jelly should be formed. The jelly is loosened by inclining and rotating the tube and the contents poured upon a filter which has previously been moistened with 0.8 per cent NaCl and attached to the funnel. A glass rod drawn out to a diameter of approximately 1.5 mm and pointed is introduced and by rotating the rod slowly the fibrin is collected quantitatively on the end of the rod as a firmly adhering mass. The fibrin is washed with distilled water from a wash bottle, dried with filter paper carefully removed from the rod by means of filter paper and introduced into a 25 x 200 mm Pyrex tube graduated at 50 c.c. One c.c. of the concentrated digestion mixture of Folin* (100 c.c. concentrated H_2SO_4 , 300 c.c. 85 per cent HClO_4 , 50 c.c. 5 per cent $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) is added, the mixture heated gently with a micro Bunsen burner until the fibrin is dissolved, then more strongly until the tube is filled with white fumes when a small watch glass is placed over the mouth of the tube and the heating continued for thirty seconds after the contents of the tube have become a light bluish shade with no trace of brown. After cooling for two minutes distilled water is added to approximately 40 c.c. the contents of the tube stirred with a long glass rod to break up any mass of silica formed and the rod rinsed with distilled water. When the contents of the tube have cooled to room temperature the volume is made up to 50 c.c. with distilled water and thoroughly mixed. The contents of the tube are now poured into a 50 c.c. conical centrifuge tube and centrifuged until the silica is all precipitated and the supernatant liquid entirely clear which usually requires from twenty to thirty minutes. Twenty five c.c. of the clear supernatant liquid are pipetted into a 50 c.c. volumetric flask. 15 c.c. of the Nessler's solution described by Folin and Wu¹² are added and the contents made up to volume mixed and compared in a colorimeter with a standard ammonium sulphate solution the standard being set at 200 mm. A 0.5 mg nitrogen standard is used which is prepared by measuring 5 c.c. of the standard ammonium sulphate solution described by Folin and Wu¹² (0.4716 gm dry $\text{C.P.}(\text{NH}_4)_2\text{SO}_4$ per liter) into a 100 c.c. measuring flask, diluting with 60 c.c. distilled water adding 1 c.c. of the concentrated digestion mixture and nesslerizing with 30 c.c. Nessler's solution. If preferred the silica may be removed by filtering through a Munktell OB or other similar retentive filter into a 100 c.c. volumetric

*The digestion cannot be carried out successfully with the dilute digestion mixture used in the N P N method of Folin and Wu as the fibrin does not dissolve readily in this mixture and during the heating becomes disintegrated and is apt to be spattered on the sides of the tube to which it adheres firmly.

flask, washing to a volume of about 60 c c, and nesslerizing with 30 c c Nessler's solution. In either case the calculation is made as follows:

$$\text{Per cent plasma fibrin} = \frac{\text{gm fibrin per 100 c c Plasma} = \frac{\text{Standard Reading}}{\text{Sample Reading}} \times 0.5 \times 6.25$$

While the agreement between duplicate determinations is good, it is always advisable to carry out two determinations on each sample of plasma and use the mean value. This can be done with 5 c c of whole blood.

SUMMARY

Details have been given of a method for the determination of fibrin in blood plasma which requires only a small volume of blood and which is believed to offer a satisfactory degree of both ease and accuracy.

REFERENCES

- ¹Meek, W. J. *Am Jour Physiol*, 1912, *xxv*, 161
- ²Starlinger, W. *Biochem Ztschr*, 1923, *cvi*, 203
- ³Cullen, G. E., and Van Slyke, D. D. *Jour Biol Chem*, 1920, *vi*, 587
- ⁴Howe, P. E. *Jour Biol Chem*, 1921, *xix*, 109
- ⁵Gram, H. C. *Jour Biol Chem*, 1921, *xix*, 279
- ⁶Foster, D. P., and Whipple, G. H. *Am Jour Physiol*, 1923, *lxiii*, 365
- ⁷Winternitz, P. *Arch Derm Syph*, 1910, *ci*, 227
- ⁸Leendertz, G., and Gromelski, B. *Arch exper Path u Pharmacol*, 1922, *cxiv*, 114
- ⁹Leendertz, G. *Klin Wchnschr*, 1923, *ii*, 746
- ¹⁰Wohlgemuth, J. *Biochem Ztschr*, 1910, *xv*, 79
- ¹¹Wu, H. *Jour Biol Chem*, 1922, *li*, 33
- ¹²Folin, O., and Wu, H. *Jour Biol Chem*, 1919, *xxviii*, 81

A DEVICE FOR THE DILUTION OF ANTIGEN IN THE KAHN PRECIPITATION TEST*

BY HERBERT SILVETTE, RICHMOND, VIRGINIA

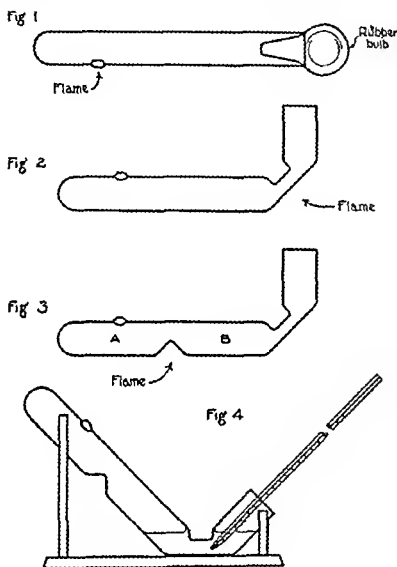
IT IS somewhat difficult without experience to dilute the antigen with saline in the Kahn Test without loss of fluid by splashing and without the separation of cholesterol crystals on account of too slow mixing. The device herein described may be used with the confidence that the dilution will be correctly made, without either of the above faults.

The device is made from an ordinary test tube, size 15 x 150 mm. The first step in the procedure is to blow a hole in the wall of the tube at a point about 2 or 3 cm from the closed end. This is done by heating the wall red hot at one point with a hot flame and blowing sharply into the other end with a rubber bulb (Fig 1). A little further application of the flame finishes off the hole nicely. Then the other end of the tube is drawn out slightly and bent sharply upwards, so that the mouth of the tube now points in the same direction as the

*From the Pathological Laboratory, Johnston-Willis Hospital.
Received for publication March 2, 1927.

opening (Fig 2) Finally, the wall of the tube is heated red hot at a point mid way between the closed end and the neck and on the opposite side of the tube from the hole The tube is quickly pressed down upon the sharp edge of a warmed three cornered file so that a partition is formed within the tube (Fig 3) By varying the height of this partition the tube may be made to dilute 1, 2, or even 3 c c of antigen

In using, an amount of antigen is placed into compartment A through the hole, and an amount of saline (as determined by titration) is gently run down



the neck into B The tube is then sharply tilted so that the saline runs into the antigen compartment, and immediately the tube is rocked back and forth a few times, thus insuring complete mixing of the contents When the hole is correctly placed, there will be no danger of the antigen dilution splashing through The apparatus must, of course be both clean and dry before using This may be accomplished by rinsing the tube first with water then with alcohol, and allowing it to drain over night

The antigen dilution after standing may be pipetted directly from the tube (Fig 4), or the contents may be emptied into a standard antigen dilution tube

NOTE ON URINE PRESERVATIVES*

BY J J SHORT, M D AND A PIATETZKY, M D, NEW YORK, N Y

THE requirements for an ideal urine preservative are summarized by Behre and Muhlberg¹ as follows

"1 It should preserve the urine from bacterial decomposition and the development of moulds or other growths for considerable periods of time under average conditions

"2 It should not interfere either positively or negatively with any of the physical, chemical or microscopic tests in ordinary use

"3 It should be readily soluble

"4 It should not interfere to any marked extent with the normal reaction of the urine

"5 It should be a solid

"6 Its cost should be reasonable "

We have conducted the search for such a preservative at varying intervals since early in 1925. Of the preservatives reported by Behre and Muhlberg we have tried boric acid, borax, toluene, thymol, resoreinol, salicylic acid, sodium benzoate, and urofix, with results very similar to theirs. It will not, therefore, be necessary to report in detail on these substances. Details of other substances and combinations we have tried follow

EXPERIMENTAL

Our tests were made on 30 cc urine specimens at incubator temperature, unpreserved specimens and specimens containing well-known preservatives were used as controls. These were examined daily for several days, and the odor, color and sediment noted. Occasionally cultures were made on blood agar plates. The specimens were also centrifuged and sediments examined microscopically where results were promising as judged by gross inspection.

Nitro-benzene, a liquid, was one of the first substances tried. This gave poor preservation alone, excellent preservation when combined with boric acid †. Only about 25 milligrams of each were necessary. It was discarded because the two substances could not be combined in tablet form.

Di-nitro-benzene, a solid, showed good preservation when combined with boric acid †. As it is only slightly soluble and leaves a sediment of its own it was discarded.

Nitro-phenol, and several other aromatic nitro compounds were tried and found unsatisfactory for various reasons.

*Experimental work described in this paper was done at the Laboratories of the Life Extension Institute, New York.
Received for publication March 1 1927.

†An additional reason for discarding boric acid is that it interferes with the Benedict picric acid sugar determination as reported by Behre and Muhlberg.

Benzoic acid alone was found to be inadequate in preserving action, although superior to sodium benzoate

Sodium bisulphite aroused our interest for some time as it met all requirements in the incubator tests. The urine was somewhat bleached but was otherwise uninterfered with and was promptly sterilized. In routine use, however, urines were not preserved due to the oxidation of bisulphite to sulphate when exposed to the air. It therefore had to be discarded.

*Chinosol** was found to be one of the most satisfactory preservatives that we have tried. About 20 mg. only are necessary for a 30 cc. specimen. It colors the urine slightly but does not interfere with any of the routine tests.

Several mercury compounds and various other substances tried by us did not give sufficiently promising results to be worth mentioning.

Hexamethylenamine—Since the paper of Behre and Muhlberg we have turned our attention to hexamethylenamine and share their opinion that it is of all tried the most satisfactory. They advised combining powdered hexamethylenamine with salicylic acid in the proportion of 3 to 2.50 mg. of such intimate mixture were then added to each 10 cc. of urine. This would be the equivalent of 90 mg. of hexamethylenamine to 60 mg. of salicylic acid for the preservation of a 30 cc. specimen. The chief objection to the preservative advocated by Behre and Muhlberg was their inability to put their mixture in tablet form. We made such an attempt and likewise failed due to the extreme bulkiness and lightness of salicylic acid. As a substitute for salicylic acid therefore we turned to acetyl salicylic acid which has quite different physical properties. Theoretically it seemed to us that this should be equal to salicylic acid for our purpose as it is more soluble in water and in contact with moisture decomposes into salicylic acid and acetic acid. Actual test proved this to be so as it gave excellent preservation when added to urine with hexamethylenamine in practically the same proportions suggested by Behre and Muhlberg for hexamethylenamine and salicylic acid. We have tested the effect of this combination on nearly all the various routine tests employed today in urinalyses and find that it interferes with only one—the ferric chloride test for acetoacetic acid. This test however is made only occasionally in routine work (when diabetes is suspected), and there can be no falsely positive report for this substance if one tries the effect of heat on the dark color produced and checks the result with the sodium utroprusside test for acetone. Although it is well known that the ingestion of coal tar products frequently causes the appearance of copper reducing substances in the urine it has been shown by Leas² that such reduction is not due to the presence of the coal tar derivatives themselves but to some excreted substance resulting from their ingestion. Sodium salicylate gave no reduction when added directly to urine in their experiment. The same was true of acetyl salicylic acid in our tests. Attempts to combine hexamethylenamine and acetyl salicylic acid in a single compressed tablet likewise resulted unsatisfactorily. Although this is possible and a good hard tablet is produced we found that the proportions of the two ingredients varied due to their difference in physical properties. Furthermore on standing the tablets became hygroscopic and dissolved after a short period of time. Finally we decided on

* A product manufactured by Parke Pharmaceutical Co., 11 West Street, New York N. Y.

the practical necessity of two tablets, one to contain hexamethylenamine and one acetyl-salicylic acid. For the preservation of 30 c c specimens we therefore make a 100 mg compressed tablet of hexamethylenamine and another such tablet containing 50 mg each of acetyl-salicylic acid and potassium nitrate, the latter an inert substance used merely for the purpose of facilitating the feeding of the acetyl-salicylic acid into the compressing machine.

COMMENT

The use of any preservative not in tablet form is time consuming. Although it would have been more desirable to have the preservative in the form of a single tablet we find that with a little practice two small tablets can be added almost as quickly as a single tablet, one tablet being added simultaneously from each hand. The substances described above are ideal for use in a tablet compressing machine as they feed regularly and give tablets of exceedingly uniform weight. Both tablets go into solution readily. The cost is extremely low when compared with that of the various urine preservatives now being marketed.

REFERENCES

- ¹Behre, Jeanette Allen, Ph D and Muhlberg, William, M D. Urinary Preservatives Including Hexamethylenamine, *JOUR LAB & CLIN MED*, May, 1926, *vi*, No 8, p 783.
- ²Leas, R D. Reducing Substances in the Urine, *JOUR LAB & CLIN MED*, October, 1920, *xii*, No 1, p 15.

THE BLOOD-URINE DENSITY INDEX*

By DAVID POLOWE, M D, PATERSON, N J

A NEW index, the Blood-Urine Density Index (B-U DI) is herewith described. It is intended to serve as a rapid orientation index of the blood-urine density balance. Less than ten minutes are required to make the determination in any given case, the falling drop method of Barbour and Hamilton¹ being used.

Only one author, Schmaltz² in 1891, so far as I have been able to discover, while determining the influence on blood density following the imbibition of great quantities of 0.6 per cent saline, made annotations as to the specific gravity of the excreted urine. He may have had some blood-urine density balance in mind but he made no comments on this phase of the matter.

BLOOD DENSIMETRY ON EXPERIMENTAL ANIMALS

Copeman,³ in 1891, ligated an extremity on each of four rabbits. The density of the blood distal to the ligature was determined. Both the red cell count and the blood density were increased in three rabbits. In the fourth rabbit, the arterial supply being also shut off, no change was found.

Popel,⁴ in 1895, working on dogs, found that the imbibition of water low

*From the Barnert Memorial Hospital
Received for publication March 1927

ers blood density, abstinence increases it while ureteral ligation plus abstinence markedly increases it.

Barbour and Hamilton, studying emotional anhydremia, have shown that " * * * excitement always increases the blood concentration, sometimes by as much as 10 per cent " This is well to remember when dealing with needle shy patients

TABLE I
COPEMAN'S COMPOSITE TABLE OF BLOOD DENSITY IN DISEASE

DISEASE	COPEMAN	QUINKE	BECQUEREL AND RADIER
Anemia chlorosis	1041-1043	1032-1049	10458 (mean of 6)
Pernicious	1027-1034		
Leucocythemia	1048-10510	10443	1036-10495 (5 cases)
Gastric ulcer	1038		
Lymphadenoma	1062		
Hemoglobinuria	10503-10520		
Scurvy		10608	
Cardiac	10495-10520	1058 (angina)	1050-10525 (55 cases)
Diabetes mellitus	10585 (2 cases)	1049-1055	
Cirrhosis of liver	10520	10496 (hemophilia)	
Acute nephritis	10570		
Chronic nephritis	10545-10600	1047-10487	
Uremia	1052	10505	
Tuberculous kidney	10485		
Tuberculous peritonitis	10570		
Typhoid fever		10544-10621	
Cerebrospinal meningitis		1059	
Pycnia		10505	
Chorea	10530		
Rheumatism and hysteria	10585		

TABLE II
BLOOD DENSITY IN DISEASE

AGE	SEX	DIAGNOSIS	BLOOD DENSITY
22	F	Tuberculosis pulmonary	10160
20	F	Tuberculosis pulmonary	10550
48	F	Tuberculosis pulmonary (flu myoma uteri)	10560
35	F	Chyluria	10450
15	F	Chlorosis	10440
62	F	Carcinoma of stomach	10390
34	F	Anemia	10580 (1)
37	M	Mitral stenosis	10510
51	F	Aortic insufficiency	10560
57	F	Syphilis	10510
30	F	Syphilis	10560
79	M	Syphilis old Senile marasmus	10520
73	F	Senile marasmus	10507
79	M	Senile marasmus	10520

From Schmaltz.

THE CLINICAL SIGNIFICANCE OF BLOOD DENSITY

This phase of clinical medicine has been neglected due possibly to the technical difficulties with the older methods. Copeman however, did a considerable amount of work along these lines and his composite table of blood density in disease appears in Table I.

Schmaltz studied the influence of large quantities of water the influence of food exercise warm baths menses age and the time of the day when the density is determined. Table II indicates his findings in diseases. He con-

cludes his paper by stating that blood density varies within narrow limits in health and varies considerably under pathologic conditions

Lyonnét,³ in 1892, cites Lloyd Jones' findings in nephritis, the blood density in parenchymatous nephritis being 1034, in interstitial nephritis 1062 to 1042. A differential diagnosis between cardiacs and nephritics is suggested in that the blood density on cardiacs is about 10594, in nephritics 10516.

Bender and Polowe,¹² in a series of eight spinal anesthetics, have found that with the fall in blood tension that follows the administration of the spinal

TABLE III*
BLOOD DENSITY AND BLOOD TENSION DURING SPINAL ANESTHESIA†

TIME A M		TENSION	FALLING TIME		BLOOD DENSITY
BP	BD		BLOOD	STANDARD	
9 57		104/72	17 7	18 6	10560
10 03	10 05	88/64	20 4		10533
	10 09		21 9		10529
10 14	10 16	84/62	21 7	18 5	10531
	10 25		23 0		10511
10 28	10 29	84/60	20 8		10527
10 35	10 36	84/64	20 9	18 4	10526
10 45	10 46	86/64	20 6		10529
	10 57		20 5		10530
10 58	10 59	154/92	20 4		10531
11 06	11 07	182/98	19 9		10535
11 08	Operation ended				

*Bender and Polowe¹²

†Case 1, October 1, 1926. Female, aged fifty. Vaginal repair. Control (prior to operation) B P 172/78 B D 10552.

anesthetic there is a concomitant fall in blood density. Table III illustrates one such case, it being noted that as the patient begins to react from the anesthesia the rise in blood tension is followed by an increase in blood density. Further data is to be collected on this phase of blood densimetry and will be published in another paper.

METHODS FOR DETERMINING BLOOD DENSITY

Three principle methods for determining blood density have been employed. These are (a) the pycnometer method, flask or capillary, the latter being used by Sehmaltz, (b) permitting a drop of blood to fall into a fluid of known density, a technique used by Hammerslag, Copeman, and more recently by Kirkpatrick and Kling,⁸ (c) the falling drop method of Barbour and Hamilton who state: "*Principle*—A drop of blood (or other body fluid) of definite size is released below the surface of a nonmiscible mixture. Its rate of fall depends on its density, which can be easily calculated as soon as the rate of fall of a similar drop of standard solution of known density (released under identical conditions) is available for comparison." For details of apparatus, material, and procedure the reader is referred to the recent publications of Barbour and Hamilton.^{1, 5}

THE BLOOD-URINE DENSITY INDEX

The density of the blood is determined by the falling drop method of Barbour and Hamilton. The density of the urine is determined in the same

way, or by urinometer, immediately before or after that of the blood is determined

The calculation of the blood urine density index (B U DI) is made by taking the values to the right of the decimal point the blood value being divided by the urine value *Example* BD = 1060 UD = 1020 then $60/20 = 3$ which is the blood urine density index (B U DI)

NORMAL VALUES

Normal Blood Densities—Fifty two observations, by the method proposed in this paper, on ten normal cases are reported in Table V These values are in fair agreement with those of Bamberger (cited by Lyonnet) and Schmaltz Normal blood densities range between 1050 and 1060, are higher in males, and higher in the morning than in the afternoon

Normal Urine Densities—It is important to note the values recorded in Table IV for the age groups between one day and fourteen years

TABLE IV
NORMAL BLOOD URINE DENSITY INDICES

AGE GROUPS	BLOOD DENSITY	URINE DENSITY	B U DI
1 to 3 days	1060-1080	1010-1012†	5-8
4 to 10 days	1060-1080*	1004-1008†	8-20
10 days to 6 mo	1053-1059*	1004-1010†	5-15
6 mo to 2 yr	1053-1059	1006-1012†	4-10
2 yr to 8 yr	1056-1060	1008-1016†	4-8
9 yr to 14 yr	1056-1060*	1012-1020†	3-5
14 yr up	1050-1060	1015-1020	2-4

Burton—From Burton Opitz *

†From Holt and Howland

BLOOD URINE DENSITY INDICES IN DISEASE

Thirty nine cases, on whom 63 observations were made are reported Fifteen cases of diabetes mellitus are recorded in Table VI Observations in one case of carcinoma of the bladder are recorded in Table VII Five kidney cases are grouped in Table VIII The other 18 cases are grouped in Table IX

Cases 8 and 9 (Table VI) were hospital cases The rest in that table were clinic patients attending the diabetic clinic in the Barnert Memorial Hospital Cases 8 and 9 were carefully dieted and insulin administered when indicated Both cases improved and with the improvement there was an increase in the B U DI, which is suggestive of the prognostic value of the index That normal urine densities may be associated with glycosuria is in agreement with the findings of Joslin¹¹ It is also of interest to note that while the B U D indices below 2 occur in about 33 per cent of the observations these are not always associated with a high urine density as might be expected from a priori reasoning Attention is also called to the hydremic condition of the blood as evidenced by the low blood densities in 37 per cent of the observations

Very low indices were found in both cases of carcinoma Case 28 was diagnosed as benign prostatic hypertrophy Concentration and dilution tests were run simultaneously with B U DI determinations The urine density fluctuations were compatible with operation The B U DI findings were decidedly against operation Cystoscopy was unsatisfactory At operation an

TABLE V
NORMAL BLOOD DENSITIES AS FOUND BY POLOWE

CASE	ROOM TEMP C	DATE	AGE	SEX	TIME*	BLOOD STAND FALLING TIME	BLOOD DENSITY	
1	23 0	8/27	33	M	A M	18 5	20 3	10573
	23 0	8/28	33	M	A M	19 5	20 3	10565
	26 5	8/29	33	M	A M	17 5	18 3	10560
	24 5	8/30	33	M	A M	18 8	20 1	10562
	22 0	9/2	33	M	P M	26 6	22 8	10534
2	23 0	8/28	23	M	A M	16 2	20 3	10600
	26 5	8/29	23	M	A M	14 6	18 3	10606
	24 5	8/30	23	M	A M	17 0	20 1	10586
	24 5	8/31	23	M	P M	20 9	19 5	10537
3	25 5	8/26	29	F	A M	21 5	18 5	10523
	24 0	8/27	29	F	A M	18 9	20 7	10565
	24 0	8/28	29	F	A M	20 5	20 0	10545
	22 0	9/2	29	F	P M	29 3	22 8	10525
4	25 5	8/26	26	F	A M	20 9	17 8	10521
	23 0	8/27	26	F	A M	23 9	20 5	10526
	24 0	8/28	26	F	A M	22 6	20 0	10530
	26 5	8/29	26	F	A M	20 7	18 3	10527
	24 5	8/30	26	F	A M	24 6	20 1	10518
	22 0	9/2	26	F	P M	30 4	22 8	10519
5	25 5	8/26	19	F	A M	18 1	17 8	10546
	24 5	8/27	19	F	A M	17 3	19 9	10550
	24 5	8/30	19	F	A M	21 4	20 1	10538
	24 5	8/31	19	F	P M	25 0	19 5	10510
6	25 5	8/26	18	F	A M	17 9	18 2	10553
	24 0	8/27	18	F	A M	21 6	20 5	10539
	23 0	8/28	18	F	A M	22 0	20 3	10536
	26 5	8/29	18	F	A M	20 4	18 3	10529
	24 5	8/30	18	F	A M	22 0	20 1	10534
	24 5	8/31	18	F	P M	25 1	19 5	10511
	22 0	9/2	18	F	P M	24 3	22 8	10540
7	25 5	8/26	28	F	A M	18 5	18 2	10545
	22 5	8/27	28	F	A M	21 5	20 5	10541
	24 5	8/28	28	F	A M	20 4	19 9	10544
	26 5	8/29	28	F	A M	18 0	18 3	10534
	24 5	8/30	28	F	A M	18 7	20 1	10564
	24 5	8/31	28	F	P M	19 6	19 5	10519
8	25 5	8/26	32	F	A M	20 1	18 2	10530
	24 0	8/27	32	F	A M	19 3	20 7	10564
	24 5	8/28	32	F	A M	18 5	19 9	10562
9	25 5	8/26	33	F	A M	19 1	18 2	10541
	24 0	8/27	33	F	A M	18 5	20 1	10565
	23 0	8/28	33	F	A M	22 7	20 3	10538
	26 5	8/29	33	F	A M	20 1	18 3	10530
	24 5	8/30	33	F	A M	22 9	20 1	10527
	24 5	8/31	33	F	P M	25 2	19 5	10510
10	25 4	8/26	18	F	A M	18 4	18 2	10547
	22 5	8/27	18	F	A M	19 9	20 7	10557
	24 5	8/28	18	F	A M	19 0	19 9	10560
	26 5	8/29	18	F	A M	22 1	18 3	10514
	24 5	8/30	18	F	A M	18 5	20 1	10567
	24 5	8/31	18	F	P M	20 0	19 5	10545
	22 0	9/2	18	F	P M	24 5	22 8	10539

* A M = Between 9 and 10 o'clock
P M = Between 2 and 4 o'clock

TABLE VI
BLOOD URINE DENSITY INDICES IN DIABETES MELLITIS

CASE	DATE	AGE	SEX	BLOOD DENSITY	URINE DENSITY	B U D I	GLAUCOMA	REMARKS
8	11/26	00	F	10536	1020	2 68	No	Insulin given
	12/6			10524	1017	3 10	No	Insulin given
0	11/27	60	F	10533	1029	1 84	No	No insulin given
	12/6			10506	1026	1 90	No	No insulin given
	12/13			10502	1022	2 24	No	Insulin given
10	11/30	40	M	10570	1037	1 54	Yes	Patient emaciated
11	11/30	40	F	10538	1015	3 59	No	Blood sugar 215
12	11/30	30	F	10543	1017	3 19	Yes	Insulin given
	12/14			10494	1020	2 49	Yes	Insulin given
13	11/30	35	F	10554	1025	2 31	Yes	Insulin given
	12/7			10544	1012	4 52	No	Insulin given
14	12/7	40	F	10510	1020	2 59	Yes	Insulin given
	12/14			10444	1031	1 53	Yes	Insulin given
15	12/7	40	F	10553	1022	1 51	Yes	No insulin given
	12/14			10412	1016	2 34	Yes	No insulin given
16	12/7	30	F	10484	1020	2 44	Yes	No insulin given
17	12/14	40	F	10318	1020	1 30	Yes	No insulin given
18	12/14	60	M	10526	1022	2 39	No	No insulin given
19	12/14	60	F	10333	1020	2 67	Yes	No insulin given
20	12/14	25	F	10392	1024	1 43	Yes	Patient obese
21	12/14	60	M	10398	1023	1 72	No	Insulin given
22	12/14	30	F	10474	1016	2 90	Yes	No insulin given

TABLE VII
CARCINOMA OF THE BLADDER*

TIME	ROOM TEMP C	URINE EXCPECTED	URINE DENSITY	BLOOD DENSITY	B U D I
8 30 A M	24.5	100 cc	1020		
9 30 A M		50 cc	1024		
10 00 A M		75 cc	1026	10360	1 39
10 30 A M		50 cc	1025	10385	1 54
11 10 A M		50 cc	1027	10353	1 31
12 00 A M		90 cc	1015		
12 20 P M				10382	2 35
2 10 P M		50 cc	1020	10398	1 99
4 00 P M		200 cc	1014	10403	2 88
5 20 P M				10433	
6 00 P M		100 cc	1015		2 89
7 45 P M		50 cc	1022	10357	1 67
8 00 A M	12/14	400 cc	1024	10350	1 46

*This renal function test used in Vienna on prostate cases was introduced in the Barnert Memorial Hospital by Dr. D. H. Mendelsohn of the Surgical Division

TABLE VIII
BLOOD URINE DENSITY INDICES IN 5 KIDNEY CASES*

CASE	DATE	AGE	SEX	BL DN	UR DN	B U D I	NPN	UN	KRYOSCOPY	DIAGNOSIS, REMARKS
23	11/24	35	M	10465	1020	2 32				Polycystic kidneys
24	12/6	40	M	10533	1012	4 44	59	27		Chr nephritis
25	11/27	35	M	10374	1012	3 12	48	24		Ac rh fev w ac neph
	12/6			10393	1024	1 63		12/10	0.59	
	12/13			10364	1026	1 40				
	12/22			10365	1011	3 32				Clinically improved
26	12/7	50	F	10288	1016	1 80				Atrophy rt k congenital
	12/13			10302	1020	1 51	26	13		Nephrectomy done
43	3/8/27	60	M	10286	1006	4 77	109	75		Chr neph, died 3/13/27

*NPN UN kryoscopy and blood sugar values in this paper were obtained from Dr. H. Wassing pathologist Barnert Memorial Hospital

TABLE IX
BLOOD URINE DENSITY INDICES IN 18 HOSPITAL CASES

CASE	DATE	AGE	SEX	BLOOD DENSITY	URINE DENSITY	B U D I	DIAGNOSIS, REMARKS
5	12/22	30	F	10326	1015	2 17	Hysteria, see anemia
7	12/22	30	F	10372	1020	1 86	Internal hemorrhoids
27	11/27	45	M	10373	1034	1 09	Ca pancreas, late
29	11/27	25	M	10430	1026	1 65	Tb pulm, effusion
	12/6			10372	1023	1 63	
	12/13			10339	1026	1 21	
30	12/22	45	M	10501	1031	1 62	Tb pulmonary
31	11/27	45	F	10509	1007	7 27	Aur fibril, decompensated
32	12/22	15	F	10497	1028	1 77	Tricus and mitral, decompensated
33	11/27	15	F	10470	1010	4 70	Rheum pneumonia, mitral
	12/6			10505	1018	2 81	Improved
34	11/27	65	F	10541	1015	3 61	Hemiplegia, chronic
	12/6			10511	1014	3 64	
35	12/6	60	F	10557	1026	2 14	Cerebral apopl coma, died
36	12/6	15	M	10537	1024	2 24	Fr skull, recovered
37	12/6	60	M	10551	1018	3 10	Prostatic hypertr, benign
38	12/6	50	M	10513	1020	2 56	Pneumoperitoncum, irritat
39	12/13	90	M	10473	1016	2.96	Senility, NPN 39, UN 22
40	12/22	30	F	10319	1019	1 68	Stric rectum, syphilis, died
41	12/24	75	M	10340	1016	2 12	Portal cirrhosis, ascites
42	11/27	40	F	10488	1014	3 49	No diag observation
44	3/10/27	45	F	10550	1015	3 67	Chronic myocarditis Kyphosis, died 3/11/27

extensive carcinoma of the bladder was found. This phase of B U DI determinations is being studied further in the hope that it may be helpful in solving the problem of operability in prostate bladder and kidney conditions.

Case 32 (Table IX) was admitted as a cardiac with mitral and tricuspid lesions, edema, and a pulsating liver. Her B U DI was below 2. This may indicate that B U DI findings in children are of greater significance when below the normal than when above it for the prescribed age.

COMMENT

The facts presented here are far too insufficient for one to draw any conclusions as to the true clinical value of the Blood Urine Density Index. At present, when this index is used in conjunction with other available clinical and laboratory facts it appears to help round out the clinical picture. Values above 4, in adults, seem to suggest intranephritic pathology. Values below 2 point toward a blood urine imbalance which seems to be primarily of extra-nephritic origin. When the B U DI hovers about 1.5 or less malignancy should be considered as a possibility.

SUMMARY

1 A new index, known as the Blood Urine Density Index (B U DI), is described.

2 The density of the respective fluids is obtained by the falling drop method of Barbour and Hamilton for blood and by the same method (or by urinometer as in the cases herein reported) for urine.

3 Normal indices for adults lie between 2 and 4. For infants and children they range from 3 to 20.

4 Pathologic indices for infants and children seem to lie below the normal for the given age group. Pathologic indices for adults lie below 2 and above 4.

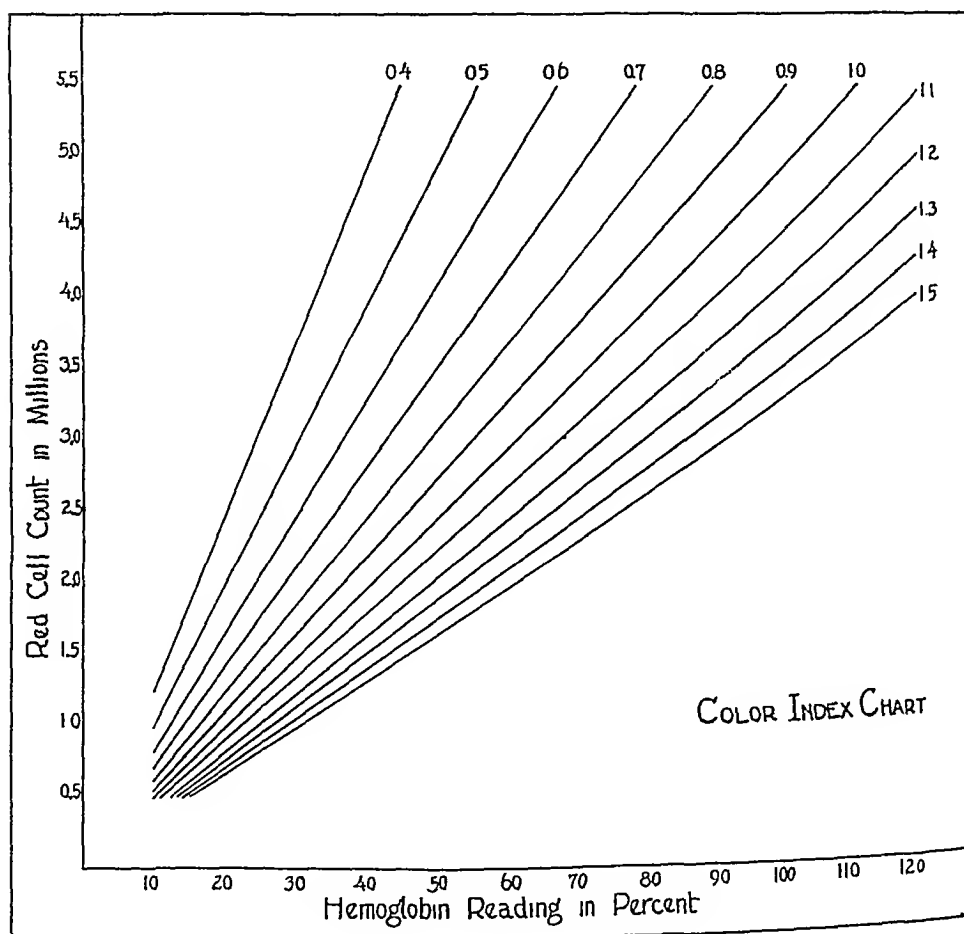
REFERENCES

- ¹Barbour H G and Hamilton W F. The Falling Drop Method for Determining Specific Gravity of Fluids. *Journal of Biological Chemistry*, August 1926, lxxix 625.
- Schmaltz von Dr Richard. Die Untersuchung des Spezifischen Gewichtes des Mnnchischen Blutes. *Deutsch Arch f klin Med* 1811, xlvii 140-158.
- ²Lyonnet Le Dr B. De La Densit Du Sang. Sa Dtermination Clinique. Ses Variations Physiologiques et Pathologiques. Paris J B Bailliere et Fils 1892.
- ³Copeman, S Monkton M A M D. Contab. Report on the Specific Gravity of the Blood in Disease. *Brit Med Jour*, Jan 24 1891, i 161.
- ⁴Barbour H G and Hamilton W F. *Jour Am Med Assn*, Jan 8 1927, lxxxviii 91.
- ⁵Popel, W. Sur les Variations de la Densit Du Sang Dans le Jeune Absolu, Simple, ou Complicue de la Ligature Des Ureteres. *Arch d Sc Biol* 1895-1896, iv 345-760.
- ⁶Barbour, H G and Hamilton W F. *Am Jour Physiol* 1924, lxxix 654.
- ⁷Kirkpatrick John and Kling David H. A Simplified Method for Determination of the Specific Gravity of a Few Drops of Urine. *Jour Am Med Assn*, August 14, 1926, lxxxvii 487.
- ⁸Holt, L Emmett and Howland John. *The Diseases of Infancy and Childhood*, ed 8, p 592.
- ⁹Burton Optiz Russell. *Textbook of Physiology* 1920, p 163.
- ¹⁰Joalin, Elliott P. *The Treatment of Diabetes Mellitus*, third edition, Lea and Febiger, p 340.
- ¹¹Bender, Theodore and Polowe David. Blood Density and Blood Tension during Spinal Anesthesia. unpublished data from the Gynecological Service of the Barnert Memorial Hospital.

A CHART FOR READING THE COLOR INDEX OF BLOOD*

By HERBERT SILVETTE, RICHMOND, VIRGINIA

THE accompanying chart was devised to make the determination of the color index of blood a simple matter. This chart is based on the formula, color index equals percentage of hemoglobin divided by percentage of red blood cells. By locating the hemoglobin percentage on the abscissa and the number of red blood cells per cubic millimeter on the ordinate, the color in



dex will be found either on one of the oblique lines, or at such a point between them that the color index can be easily and accurately estimated to 0.01

There seems to be no such chart as the one above described in use at the present time. By means of it the color index may be read directly and the procedure should be routine in connection with red blood counts and hemoglobin estimations.

*From the Pathological Laboratory, Johnston-Willis Hospital, Richmond, Virginia.
Received for publication Jan. 24, 1927.

A SIMPLIFICATION NOT A MODIFICATION OF THE KOLMER TEST*

By JAMES B. RUCKER, JR. A.B. M.D. TOLEDO, OHIO

SO MUCH has been dished up for the consumption of medical readers during the past five years concerning the standardization of the Wassermann test for syphilis that it may seem rather presumptuous on my part to again stir up the same old hash and attempt to feed it to you with the same old spoon by simply mixing it together a bit more and warming it over. I know a little too well however from my own personal experience, that you could not be expected to swallow such a warmed over concoction and pretend to relish it.

I am frank to say that I do not intend to urge any new method for the standardization of so important a test as the Wassermann for we have a perfectly satisfactory one already. We all are agreed by this time that the most excellent method worked out by Kolmer and his assistants over a period of many years of painstaking effort has given us a method far superior in its results in eliminating inaccuracies to any which has thus far been devised.

It has been shown in thousands of tests that its delicacy in detecting exceedingly small amounts of syphilitic antibody in the blood or spinal fluid is as great as could be desired without any tendency to err on the positive side. To attempt to devise a method of greater sensitivity than that set forth in the Kolmer test would be to condemn many a patient to untold anxiety concerning a disease which he never had or if he did have, of which he is now free so far as is shown by the production of syphilitic antibodies in the tissues of the body and their circulation in the blood stream.

On the other hand if a patient has not a syphilitic infection the exceedingly well balanced technique of the Kolmer test wherein the amount of amboceptor is delicately adjusted to the complement used and the amount of complement just as delicately adjusted to the amboceptor immediately before each series of tests is run the negativity of the reaction comes out strong and clear, without any equivocation and to one who has run a sufficient number of these tests to have learned its perfect reliability there is the supreme satisfaction of absolutely knowing that this patient's blood is free of any luetic taint. In other words the Kolmer test picks up all the positives of whatsoever degree of which there may have been some doubt by any other test, and is especially valuable in treated cases where the physician is anxious to know what, if any progress he is making in freeing his patient of the luetic infection but never, throughout my experience of more than four thousand tests has it given a positive reading when the

*Read at the Seventh Annual Session of the Ohio Society of Clinical and Laboratory Diagnosis at Columbus, Ohio, May 1st 1927

family and personal history, thoroughly delved into, and clinical evidence in the patient himself, presented negative findings

If, then, it is recognized and agreed by a large majority of clinical pathologists that the Kolmer test is the most reliable complement fixation test yet devised for the detection of the syphilitic antibody in the blood or spinal fluid, why is it, that we do not all adopt it as the standard method of performing the complement-fixation test for syphilis in our laboratories and use it to the exclusion of any other such test as a basis for reporting our results in what is still wont to be called the Wassermann test? Or, better still, why not report all our results of the complement fixation for syphilis as negative or positive (of whatever degree) Kolmer tests, for Wassermann long flourished in his day, and now deserves a well-earned rest?

Kolmer in his foreword in his articles in the *American Journal of Syphilis* and other publications, wherein he gives the technic of his standard test in the clearest detail, has struck at the root of the matter and has given us one answer to my question when he says that in his long search for a standard method he has evolved one which although the most accurate and reliable in its freedom from error on one side or the other, that has yet been offered, it is still more time-consuming than most of the technics at present in use, and advises those who are willing to sacrifice accuracy of result to rapidity of performance in seeking a short cut in serologic diagnosis, to scrupulously avoid his test, for such is not for them, and will lead only to disappointment

Another reason, and the main one I think, that many clinical pathologists have not adopted Kolmer's modification as the one exclusively used in their complement-fixation tests for syphilis, is not that the result may not be reported for twenty-four hours after the test has been begun, but that the test seems to some, unreasonably complicated with the "set up" of its six tubes for each serum, the antigen control, the corpuscle control, and the reading scale for each series. This need not greatly worry one, however, inasmuch as this becomes comparatively simple when one has, after some experience, become accustomed to the various details. Nevertheless, in view of the fact that the great reliability of the Kolmer test had been so strongly impressed upon me by a great deal of personal experience with it in its original form it occurred to me that if I could simplify the number of manipulations of the test itself, without in any way modifying its essentials, its adoption as the exclusive complement-fixation test for syphilis by the smaller hospitals and private clinical laboratories, would be greatly furthered.

Consequently, in my first attempt at simplification, I eliminated the reading scale, after having used it for some time, and having found its use added nothing to the interpretation of the reading of my results. My readings of the results of the tests themselves without the reading scale's aid were precisely the same as they were with it. I had always felt that the setting up of the scale was superfluous in the Kolmer test, inasmuch as it had seemed to me to be based upon a wrong theory of the manner by which the results were to be interpreted, because, the quantitative result as regards the degree of positivity does not depend upon the degree of fixation of complement in any individual tube dilution of the serum under examination, but upon the ability

to determine whether or not there is even the *slightest* amount of fixation in any individual tube dilution in the series. Kolmer specifically states in regard to the interpretation of the reaction, that a *very strongly positive* reaction is indicated when there is *partial* or complete fixation in the first four or all five tubes, *strongly positive*, when there is *partial* or complete fixation in the first three tubes, *moderately positive*, when there is *partial* or complete fixation in the first two tubes, *weakly positive* when there is *partial* or complete fixation in the first tube only, and *negative* when *all* tubes show complete hemolysis. Again, in a later paper, in regard to interpretation he says: "When there is fixation of any degree in the first four or all five tubes—and so on. Therefore from these citations we see that in describing the manner of interpretation of the test he dwells particularly on the words *partial* or *complete* fixation and fixation in any degree."

If, then, the *degree* of fixation in the individual tube is unimportant according to Kolmer, why should the test be complicated by consuming additional time and labor involved in setting up a reading scale for comparison when the reading of the results of the test itself depends not at all upon the *degree* of fixation or hemolysis in the individual tube dilution but upon the fact as to whether or not fixation has occurred in *any degree* whatsoever? Personally, I can see no good reason for its retention as a part of the Kolmer Qualitative Test, and although I discarded it some three or more years ago I am quite satisfied that my results have been as good since that time as they were when I was still using it. This has I feel eliminated quite a good deal of time and labor and has considerably simplified the quantitative test.

I worked along for some time without further elisions in the test until one day the thought occurred to me that if the first four tubes only or if all five tubes, show partial or complete fixation the result is the same—*very strongly positive*. Why, then, shall I not eliminate the fifth tube?

In the several thousands of tests performed I had never secured fixation of any degree in the fifth tube when such fixation was absent in the fourth tube, although I had oftentimes secured fixation of some degree in the first four tubes, with complete hemolysis in tube five. The conclusion naturally to be drawn, was that, if fixation occurred in any degree in the first five tubes, or if it occurred only in the first four tubes and the fifth tube showed complete hemolysis, the result was the same *very strongly positive*, therefore the use of the fifth tube was superfluous. I am not fostering a political machine in my laboratory, so when one of my heretofore supposedly useful workers failed to show cause why he should be retained on the rolls I dismissed him—and out went the fifth tube with no modification, so far as I could observe, of the delicacy or accuracy of the Kolmer Quantitative Test. Of course, if fixation should ever have occurred in the fifth tube and not in the fourth, which has never happened in my hands I could not have reported the result as *very strongly positive*, but would have felt that my technique had been in error and that such an occurrence demanded a new set up of the serum in question and a repetition of the test.

A third simplification is that step wherein after the second incubation has been completed and the results have been read, instead of placing the

positive sera in the ice box for three hours to settle, after which time, they are taken out and a second reading made, I have for the past two years, been placing the positives in the centrifuge and allowing them to centrifugate at high speed for three minutes, after which the second reading is made. This markedly shortens the time employed in the original test, and gives no difference in my results, so far as my observation goes. The hour's incubation in the water-bath should hemolyze all the cells that are going to be hemolyzed, provided one's reagents are properly adjusted as outlined by Kolmer, before the test itself is begun.

Unless they are, it is the height of folly to begin the main test, with the expectation that the results will be anything short of disastrous. Therefore it has seemed to me that when one means of precipitating the cells which have not been hemolyzed, is as good as another, the shorter in point of time, should be the one chosen, especially if it tends to render the test more practicable, and for that reason, more readily adopted as a Standard Quantitative Test.

As for the corpuscle control and the antigen control, it is immaterial whether one uses them or not—just as one chooses. With my known negative serum and my known positive serum controls which I always use in the set up of each series, I do not feel that they are at all necessary, and I never use them.

With more than 4000 sera I have used the Kolmer Quantitative Method simplified in the manner which has been described, and feel that while the method worked out by Kolmer has been carefully adhered to in all its essential points, I have simplified it considerably both in economy of time and labor by cutting out some of the nonessential details, so that it takes very little more time than the older Wassermann technique, with its two or three antigens, and it is far more satisfactory in its results. When I get a Kolmer, 4, 3, 2, 1, plus or a negative now, I feel that it is absolutely right, the last word has been said. Whereas, in using the old Wassermann method, I oftentimes was perplexed to determine whether my result was a 3 plus or a 2 plus or a 2 plus or a 1 plus. Now, with the Kolmer test so simplified, I am confident when I state the *degree* of positivity.

I tritrate my complement and amboceptor in the afternoon and set up my series of tests at the end of the day just before leaving my laboratory, put them in the ice box, and the next day at noon, treat them with amboceptor and sheep cells, and put them in the water-bath for an hour. Three minutes in the centrifuge for the positives completes the process, and they are ready to be reported. Thus performed, it is exceedingly practicable in my own small laboratory, and I am sure it will be in yours. Try it. In comparison, in satisfaction as to accuracy of results, there is no equal to the Kolmer Quantitative Test.

A METHOD FOR THE DETERMINATION OF THE COAGULATION TIME AND REFRACTION TIME OF THE BLOOD*

By J W SOO¹, M D AND THEODORE S MOISE, M D NEW HAVEN, CONN

THE object of this communication is to present a simple capillary tube method for the determination of the coagulation time of the blood which has the advantages of utilizing a minimal quantity of blood and allows a reading of the time of complete retraction of the clot. The advantages of the latter determination are too obvious to require any discussion at the present time.

A discussion of the factors concerned and the various methods of estimating the coagulation time is also unnecessary as they have been very thoroughly reviewed in papers by Hinman and Sladen (1907) and by Cohen (1911). The latter author has discussed thirty one methods and modifications for the determination of the coagulation time of the blood.

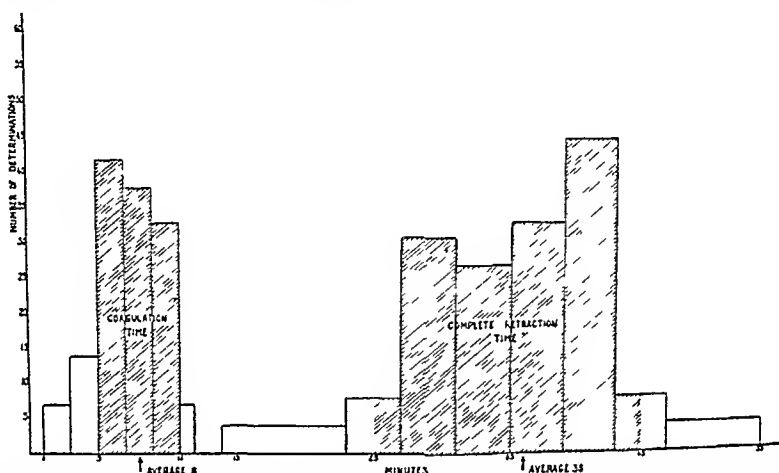
Among these methods are ten in which the blood is drawn from a small puncture wound into capillary tubes. They are all modifications of one of the four following methods. (1) *Vierodt's*. The adhesion of fibrin threads to a white horsehair after withdrawal from the capillary tube indicates the beginning while the completion of coagulation is indicated when the coagulum no longer adheres to the thread. (2) *Wright's*. The investigator blows through the blood containing capillary tubes at different intervals when the blood cannot be dislodged, it is called 'clotted'. Other stages are designated by the terms 'liquid' or 'clotting'. (3) *McGowan's*. The end of the capillary tube is broken off at different intervals and the first stage of coagulation is indicated by a minute shred stretching between the broken ends. Care must be taken to keep the broken ends approximated until the reading is made. (4) *Schultz's*. This method utilizes a capillary tube four inches long blown out into a number of regular expansions forming tiny bulbs with short spaces between. The tube is filled with blood and at definite intervals a bulb is broken off and shaken in physiologic salt solution. The end point is designated as the time when the bulb remains filled with clot and only a few red blood cells drop into the solution.

All of these methods have been unsatisfactory in Cohen's hands on account of their variable readings. He concludes that the most accurate is Addis' modification of Brodie and Russell's method which requires a special instrument and is too intricate and cumbersome for general use. Among the methods that he has reviewed Milhan's is the simplest. This method consists in allowing a drop of blood to fall upon a glass slide, which is tipped vertically at frequent intervals. The coagulation time is read when the drop does not change its shape and maintains its convexity of outline. Cohen

From the Department of Surgery, Yale University School of Medicine.
Received for publication June 17 1911

believes that his own modification of Milhan's method is the best one available. This modification (Cohen's) consists of apparatus devised to prevent evaporation and maintain a constant temperature and has the disadvantage inherent in any method requiring special apparatus.

Lee and White (1913) have described a method for which they claim the advantages of simplicity and reliability. In this method 1 c.c. of blood is withdrawn by vena puncture with a small sterile glass syringe which has been rinsed with physiologic saline. The needle is removed and the blood emptied into a small clean glass tube which has also been rinsed with physiologic saline. The time at which the blood is withdrawn is accurately noted. The tube is rotated endwise every thirty seconds and the end point is read at the time at which the blood no longer flows but maintains its surface contour when the tube is inverted. Among the advantages described for this method are that the blood is obtained without contact with the skin or other tissue and that a sufficient amount of blood is obtained to study the charac-



The chart shows the results of a series of determination on normal individuals for the purpose of standardizing the method. The shaded portions indicate the time intervals within which a large majority of determinations of the coagulation time and time of complete retraction fall.

ter, color and retraction of the clot. This method has the disadvantage of requiring a vena puncture to obtain the required amount of blood.

The method to be described differs in principle from the older capillary tube methods, utilizes a minimum amount of blood, and affords the additional opportunity of studying the retractability of the resultant clot.

METHOD

Preparation of Capillary Tubes—Glass tubes of convenient length and about 5 mm. in diameter are thoroughly cleaned, dried and carefully protected from dust. Capillary tubes about 0.3 mm. in diameter are drawn and cut into 3 cm. lengths. These tubes are measured with a micrometer and only those with an inside diameter varying between 0.2 mm. and 0.3 mm. are selected.

Drawing the Specimen of Blood—The skin is washed with alcohol and ether, care being taken to have the area quite dry. The puncture is made of

sufficient depth to give a free flow of several drops of blood. The tube is held with forceps and filled by capillary attraction from the first drop of blood. The tube is placed on a clean glass slide and observed with low power magnification using an ocular micrometer.

Observation of the Specimen—The time of the puncture is recorded and the following changes are observed: (1) A thin opaque line appears between the blood and the walls of the tube (average time of occurrence in normal individuals two minutes and forty-four seconds). There is a gradual appearance of a serrate outline to the marginal red blood cells and the opaque streak becomes wider (average time of occurrence in normal individuals four minutes and fifteen seconds). (2) The blood begins to retract from the wall of the capillary tube. This has been taken arbitrarily as the end point for the 'coagulation time'. The average time for normal individuals is eight minutes. (3) The retraction proceeds for approximately thirty-five to forty-five minutes and is best observed by adjusting the micrometer so that a mark coincides with the retracting clot margin. The change is noted at frequent intervals. The interval from the moment of puncture to the instant at which no further retraction occurs is designated the time of complete retraction. The average retraction time for normal individuals is thirty-six minutes.

DISCUSSION

The method was devised by one of us (J W S) for use in experimental studies on white rats and has been utilized in the clinic in our studies on the treatment of purpura hemorrhagica by exposure to the mercury vapor quartz lamp (Sooy and Moise 1926). The procedure has been standardized by performing approximately 150 determinations on fifty normal individuals. The results are given in the accompanying graphic chart which shows that a very large majority of the determinations fall between five and eleven minutes for the coagulation time and between twenty-five and forty-five minutes for the time of complete retraction. The averages for these determinations are eight and thirty-six minutes respectively. The determinations were done at temperatures ranging from 20° to 25° C.

The chief advantages in the method are the utilization of minimal quantities of blood and the opportunity it affords for observations upon the retractability of the blood clot. On the other hand its chief weakness is that the method requires a certain amount of practice in learning the exact end point for determination of the coagulation time.

REFERENCES

- Cohen M S Arch Int Med, 1911, viii 684
 Hinman F, and Sladen, F J Bull Johns Hopkins Hosp 1907, xviii 298
 Lee R. I and White P D Am Jour Med Sc 1913 cxlv 494
 Sooy, J W, and Moise, T S Jour Am Med Assn, 1926, lxxxvi 94

A VARIABLE FILTER FOR THE MICROSCOPE*

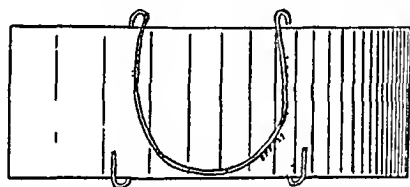
BY ROY F. FEEHSTER, M.D., D.P.H., LOUISVILLE, KY

SLIDES stained differently often need more or less of the red rays filtered out to obtain the best results when examining with artificial light, but very few microscopes are equipped with any kind of filtering apparatus except a blue glass disc.

The writer has tried a number of filters but the disadvantage of most of them is that the amount of blue cannot be varied. Some time ago he devised one which had this desirable feature. It has proved exceedingly satisfactory and has the added advantage of being very simply made.

The filter consists simply of a strip of photographic celluloid film, about $1\frac{1}{4}$ inches wide by $3\frac{1}{2}$ to 4 inches long, which is stained heavily with methylene blue at one end and shades off to almost colorless at the other end. This strip is slipped under the ring which usually holds the filter disc to the substage condenser. It can then be moved to the right or left as desired, thereby giving varying amounts of blue.

Many microscopes, instead of having the hinged ring to hold the disc, have a slot into which it is slipped. In this case a wire attachment, like that shown in the accompanying illustration, must be made to hold the filter under the



substage condenser. The curved portion of the wire is slipped into the slot and the filter can be moved to the right or left.

I obtain large sized films from the X-ray laboratory and remove all of the gelatine emulsion by treating with hot water. The filter is best colored when making several at a time, that is, cut off a strip of the film about $3\frac{1}{2}$ inches wide, lay it on the table and wet the surface with a damp cloth. Then take a small brush and apply methylene blue dissolved in wood alcohol, beginning at one side and gradually working toward the other, carrying the brush from end to end in long sweeping strokes. Dip the brush repeatedly into the methylene blue solution and repeat the operation. The wood alcohol in high strength softens or dissolves the surface layer of the celluloid and the methylene blue is precipitated into the film. The gradual decrease in the strength of the alcohol, as diluted by the water applied in the beginning, insures a gradually diminished intensity of color. After the strip has been stained properly it is cut into smaller strips about $1\frac{1}{4}$ inches wide. The methylene blue gradually fades, so all except the filter in use should be put away in the dark.

This filter makes it possible to have a high degree of illumination without the usual eye strain. Students in pathology here who have been given these filters are vastly pleased with the added comfort and the ease in distinguishing structures.

*From the Department of Bacteriology and Pathology of the University of Louisville School of Medicine.
Received for publication May 19 1927

CEDAR OIL AS AN AID IN FINDING PARASITIC OVA IN FECES*

BY GORDON E. HEIN, M.D. SAN FRANCISCO, CALIF.

FOR several years, at the San Francisco Hospital we have been using a method in searching stool specimens for parasitic ova which we feel deserves a more widespread application.

It depends upon the property of cedar oil in clarifying smears of dried

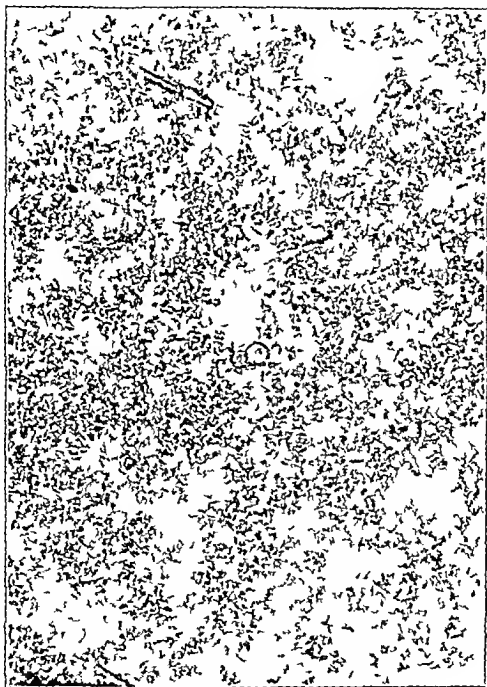


Fig. 1.—Fresh feces showing a single egg.

feces so that very thick smears may be utilized in looking for ova, with greatly increased probability of finding them.

An extremely thick smear of the suspected feces is made upon a slide and allowed to dry at room temperature. The thickness of the smear is

From the Department of Medicine, University of California Medical School.
Received for publication June 1, 1937.

about from five to ten times as heavy as ordinarily would be used, and a few trials will show the approximate thickness necessary

Cedar oil is dropped upon the field and covered with a cover glass. The feces are rendered transparent and ova are greatly accentuated by the clear background.

The slides may be kept for some time and we have ova of *trichocephalus trichiura* which are unchanged after three years.

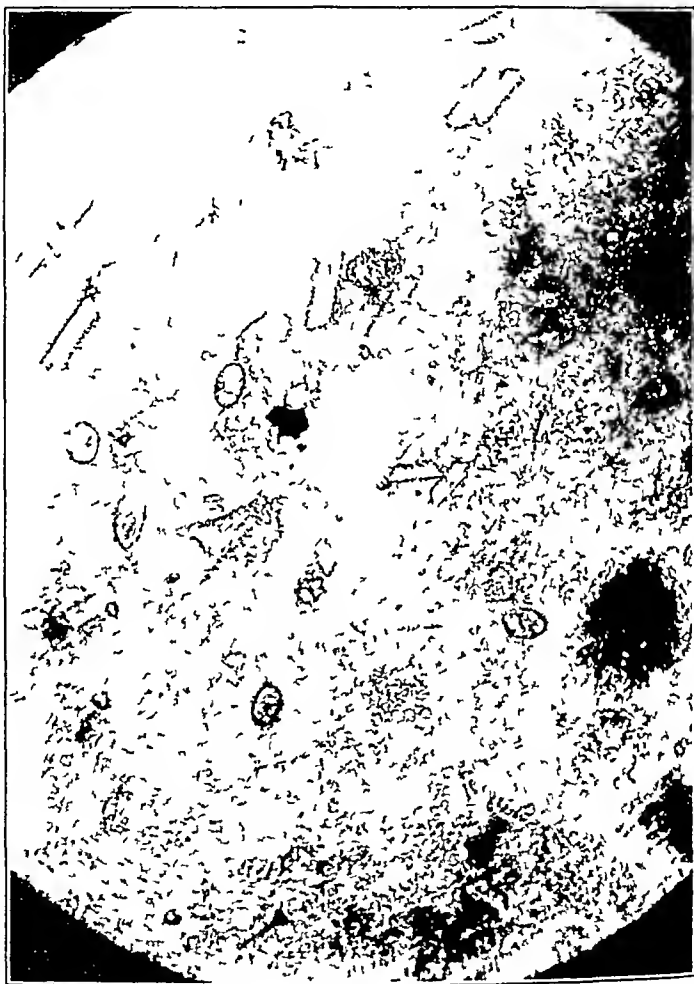


Fig 2—Dried smear clarified by cedar oil. Background much clearer in spite of thickness of smear.

Fig 1 shows a fresh smear of feces in which a single hookworm egg was found after prolonged search. A thick smear clarified with cedar oil showed six ova to one field (Fig 2).

By this method we have found ova in feces without difficulty where repeated search of fresh smears without concentration methods failed to show them and, without doubt it could be used to supplement other concentration methods such as centrifuging or the brine flotation method of Kofoid and Barber. The simplicity and ease with which ova are revealed seems to us to advocate the method strongly.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D. ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

LYMPHOBLASTOMA Lymphoblastoma Aspects Concerning Abdominal Lesions Especially Their Production of Early Symptoms Minot, G. R. and Isaacs, R. *Am Jour Med Sc*, August 1926, class, No 2 p 157

Twenty five per cent of 477 patients (401 dead 76 living) with lymphoblastoma, exclusive of lymphatic leucemia, had as their initial symptom one referable to an abdominal lesion. Twenty per cent of the deceased without such initial symptoms had symptoms referable to abdominal disease early in the course of their illness. Late in the disease such symptoms occurred with very great frequency.

Symptoms due to lymphoblastoma within the abdomen are protean and may simulate many kinds of disorders. They often lead to an incorrect diagnosis but need not necessarily do so if the condition be recalled and all facts evaluated properly.

Abdominal and back pain, often intense pains in the legs and various gastrointestinal symptoms may be prominent early ones. Genitourinary symptoms, jaundice, signs of invasion of the pancreas, adrenals, and spinal cord are apt to appear late. Fever and skin manifestations may occur early and even be initial when the chief lesion is in the abdomen. Pregnancy may run its normal course in patients with lymphoblastoma and is not unusual.

In but 27 per cent of the 119 cases with initial symptoms referable to abdominal lesions, was there enlargement of lymph nodes in the groins early and at this time only 23 per cent had enlargement of other external nodes.

Cases of this sort occur more often in the later than the earlier years of life, even so, cases with early, but not initial symptoms referable to abdominal lymphoblastoma occur particularly in the third decade of life.

After the appearance of symptoms referable to an abdominal lesion the prognosis for a long duration of life is worse than when such manifestations are absent.

Irradiation of abdominal lesions can alleviate markedly these patients' symptoms. It has not prolonged definitely, on the average, the duration of disease but probably occasionally can do so. Likewise, rarely the duration of a case may be extended by a radical surgical operation.

PERITONITIS MERCUROCHROME IN The Treatment of Experimental Peritonitis by Mercurochrome—220 Soluble, Wiles A. M. and Haskell C. C. *Arch of Surg*, May 1926, xxi, 1080

A series of experiments upon dogs in which experimental peritonitis was produced by incision of the large bowel. The animals were treated by the intravenous injection of 5 mg of mercurochrome in 1 per cent solution per kilogram body weight. In every instance in which the animal survived more than twenty four hours this dose was repeated daily until death or apparent recovery was manifest.

While slightly beneficial results were noted in twenty five animals no spectacular effect was seen and, in the endeavor to bring this out a further series received in addition to the mercurochrome, 10 c.c. of 25 per cent glucose per kilogram.

The dextrose treated animals showed a higher mortality than those treated with mercurochrome alone, possibly because the hypertonic dextrose solution hastened absorption from the peritoneal cavity.

The authors are not enthusiastic about the value of mercurochrome in peritonitis, recognizing, however, that peritonitis is a toxemia as well as a bacteremia.

RHEUMATIC FEVER Rheumatic Fever An Analytic Study of Three Hundred and Ninety-three Cases of Rheumatic Fever and Eighty-nine Cases of Chorea, Mackle T T Am Jour Med Sc, August, 1926, clviii, No 2, p 199

Rheumatic fever in approximately 70 per cent of all cases, irrespective of age, presents itself as a chronic disease, characterized by periods of recurrence of the acute features—fever, arthritis and leucocytosis. The age of the patient is a highly important factor in the prognosis. This is evident from the fact that under the age of fifteen years is found the highest incidence of first attacks of cardiac involvement and of liability to recurrence. Serious cardiac involvement occurs in 68 per cent of cases irrespective of age. Between the ages of ten and fifteen years approximately 78.2 per cent of all cases present evidence of this complication in the first attack, and only after the age of twenty five years does the incidence of heart disease fall below 50 per cent in the initial attack of rheumatic fever.

That focal infection plays a rôle in the etiology seems apparent from a comparison of its occurrence in the rheumatic cases, with a series of 400 nonrheumatic controls. In the former group it was found in 80 per cent of individuals as against 66 per cent in the latter group. Tonsillar infection was found to be more than twice as prevalent in the rheumatic fever cases as in 400 nonrheumatic controls. The complete removal of the tonsils when evidence of infection is present, together with appropriate treatment of other foci of infection seems to reduce but not to remove the incidence of recurrences of rheumatic fever.

The expected incidence of recurrences of all age groups was found to be 71 per cent. In patients above the age of twenty years, at the time of the first attack of rheumatic fever, it was found to be 58.6 per cent, while below the age of twenty the incidence rate was 78.2 per cent. In a general way, the younger the patient at the time of the first attack of rheumatism, the greater the probability of recurrences, 93 per cent of all cases having the first attack between the fifth and tenth year have recurrences of the acute condition.

Only 57 per cent of the first recurrences were found to develop within a period of four years following the first attack of rheumatic fever. This would seem to be a very important factor in analyzing the true worth of any therapeutic or prophylactic attack upon the problem of rheumatism.

DIABETES Constitutional and Hereditary Traits in Diabetes, Borach, J J Am Jour Med Sc, August, 1926, clviii, No 2, p 243

A correlation of observations upon 350 cases

Nutritional History Recorded at four periods

A At maturity—21 years 100 cases, weight normal

B Maximum weight and age at which attained between the age of 21 and the time diabetes was discovered, the average weight increase was 59.5 pounds

C The time when weight began to diminish

D The weight at the beginning of treatment

For the series, 90 per cent were obese

Dietetic History A history of overeating was found in only 20 per cent

Height and Weight No characteristic deviation from normal, other than excessive fat deposits, was encountered

Complexion Light complexion is very characteristic and an element in the composite picture of the typical case of diabetes

Hirsuties and Skin The typical diabetic is light complexioned, has a scant covering of hair over the body, and the skin is abnormally smooth

The thyroid gland is frequently involved and skin lesions, such as papillomas, angiomas, xanthomatous plaques, and diffuse brownish pigmentations are not infrequent

Hereditary Tendency A definite familial history was encountered sixty-eight times. If it cannot be said that an individual having these stigmata is predestined to become diabetic, these observations do justify us in saying that in diabetes they occur with striking coincidence. We look upon the stature, obesity, coloring, lesions of the skin, endocrine disturbances, family history of diabetes and the other findings to which we have referred as in

dicating constitutional disturbances, which make an individual diabetic. They seem intimately concerned in the cause and the effect of the disease. An individual who presents these findings, is the kind of an individual who develops diabetes.

CHOLERA INFANTUM Upper Respiratory Infection as a Cause of Cholera Infantum, Jeans P C and Floyd M L. Jour Am Med Assn, July 24 1926 lxxxvii, 220

Observations of the authors lead them to believe that there is a relationship between upper respiratory infection and a clinical picture corresponding to what has been described under the term cholera infantum. In recent years all patients presenting this clinical picture who have come under their observation have had either mastoiditis or paranasal sinusitis or both as the apparent underlying cause of their disturbance. The infection is seldom obvious, while the gastrointestinal symptoms are usually prominent. The establishment of adequate drainage from the site of infection brings about prompt and complete recovery.

INSULIN Blood Sugar Content and Insulin Treatment of Dermatoses Simon F Arch f Verdauungskr, 1926, xxavii, 363

According to the author a marked increase in blood sugar value occurs in psoriasis and in furunculosis. In cases of psoriasis higher values are said to be noted in males than in females, while in furunculosis the increase is declared to vary within somewhat narrow limits.

Blood sugar value is said to exhibit an increase in the majority of cases of eczema, and to vary within wider limits. Highest values were encountered in relapses and in chronic cases, and relatively high values in eczema in diabetes mellitus.

Insulin treatment of these dermatoses which are accompanied by increase in blood sugar value, is declared to lead first to a temporary increase and later to a decrease in blood sugar value. Thus, it is pointed out pruritus is prevented and predisposition of the skin to injuries due to activity of bacteria is diminished.

Simon is of the opinion that insulin may through its increase in the alkalinity of the blood exert a favorable effect upon many diseases of the skin.

BILIRUBIN The Formation of Bilirubin Mann, F C Sheard C and Ballman J L Minn Med, May 1926 227

The investigations of the authors lead them to conclude that bilirubin is formed from hemoglobin and that hematin appears as an intermediate product in the formation of bilirubin from hemoglobin.

MEASLES Measles Prophylaxis Use of Blood from Convalescents in a School Epidemic Townsend J H Boston Med and Surg Jour May 13 1926 cxvii No 19, p 869

The use of prophylactic measures in an epidemic of 63 cases of measles in a boarding school of 400 boys is described.

In a dosage of 20 cc the blood from an adult who had measles twenty years previously seemed to have no effect either in preventing or modifying the disease.

Blood from convalescents in a dosage of 9 cc of whole blood (0.5½ cc serum) had little or no effect in preventing infection but influenced markedly the course of the disease when it was given before the end of the first week of the incubation period.

Thirty-two cases who received convalescent blood at least eight days before the development of the rash showed an average duration of the febrile period of 3.66 days, whereas 21 boys who received no inoculation showed an average duration of the febrile period of 6.43 days.

The average maximum temperature of the 32 who received convalescent blood at least eight days before the development of the rash was 102.3 F whereas the average maximum temperature of the 21 who received no inoculation was 102.5 F.

The average stay in the infirmary of the 32 inoculated boys was 9.1 days whereas the average stay of the control group was 13.0 days.

No complications whatever occurred in the inoculated group of 32, whereas in the control group of 21 there was one case of bronchopneumonia, one of otitis media, one of frontal sinusitis, and one of external otitis

The mild character of the disease in many of the boys who received inoculations was very striking

The inoculations had no ill effects whatever

Beneficial effects were obtained whether the blood was administered as late as 6 days after exposure or as early as twelve days before the probable date of infection

THYROID DISEASE Calcemia and Glycemia in Thyroid Diseases with Increased Basal Metabolism, Waldorp, C P, and Trelles, R A. Rev Soc Argentina de Biol, December, 1925, 1, 762

The following conclusions are drawn from a study of twenty six cases

- 1 Hypocalcemia occurred in all cases with a basal metabolism above normal
- 2 There was no absolute parallelism between the calcemia and the basal metabolism.
- 3 In half of eighteen cases the blood sugar was increased

HEMOPHILIA Blood Clotting Studies in Hemophilia, Mills, C A. Am Jour Physiol, May, 1926, LXXVI, No 3, p 632

Very fresh serum, obtained by clotting hemophilic plasma with tissue fibrinogen, is found to contain a rich supply of active thrombin, but no prothrombin capable of activation of cephalin.

This thrombin very rapidly disappears from such serum, and at no time in the aging process can any new thrombin be produced by cephalin such as we see in normal serum.

Cephalin in one case actually delays, and in the other case only very slightly accelerates the clotting of recalcified hemophilic citrate plasma. This together with its inability to act on hemophilic serum, leads us to believe that the fault lies in some fashion in the prothrombin factor

There is found no increase in the antithrombin of hemophilic blood or serum.

Protein sensitization and local skin reaction in a hemophilic generates a normally reacting prothrombin in the blood and increasing the cephalin effect on the plasma and serum

Tissue fibrinogen clots such blood equally well whether normal prothrombin be present or absent, confirming our views as to the two independent clotting processes

Witte peptone preserves the thrombin of hemophilic serum and enables cephalin to exert its characteristic action

DIABETES Necropsy Findings in Diabetes, Wilder, R M Southern Med Jour, April, 1926, XIX, No 4, p 241

An analysis is presented of the pathologic conditions found in a group of eighty one fatal cases of diabetes. Diabetes was solely and directly responsible for death in ten cases, death in the remainder being due to degenerative complications or to the consequences of operations. Necropsy was performed in fifty eight cases.

Gallstones were found in sixteen of the fifty eight cases at necropsy. Pancreatic stones were found in three cases. Sclerosis of the kidneys occurred in fourteen instances, and chronic diffuse nephritis in four.

Arteriosclerosis of considerable degree occurred in nearly all cases when the age of the patient exceeded forty years.

Gangrene accounted for fourteen of the eighty one deaths and was associated with a high degree of coronary sclerosis and myocardial damage in 75 per cent of cases in which the heart was examined at necropsy.

A very high incidence of advanced coronary sclerosis was encountered in the series as a whole (seventeen cases among fifty eight), associated usually with marked fibrosis of the myocardium.

In four cases exophthalmic goiter and diabetes were combined. In two of them the pancreas revealed little or no anatomic abnormality.

Hydropic degeneration was not recognized but marked fatty changes in the islands occurred in eleven cases

In several cases representing the most severe and intensive instances of diabetes in the series, the pancreatic lesions were trivial. On the other hand severe pancreatic lesions were frequently found in cases of relatively mild diabetes in which death was due to degenerative complications

A parallelism between the degree of parenchymatous changes and the intensity of the clinical symptoms of diabetes does not exist. The explanation of the cause of diabetes must involve considerations such as heredity of predisposition and ability of cells other than those of the pancreas to elaborate insulin

SMALLPOX The Blood in Smallpox During a Recent Epidemic Ikeda K Arch Int Med, May, 1928 LVIII, 660

An analysis of 250 examinations of 200 cases

The blood of smallpox shows characteristic findings which if properly interpreted are of definite diagnostic and prognostic value

The earlier the rise of the platelets the sooner the approach of the desiccation period prognosticating a shorter course of the disease

A definite leucopenia during the maculopapular stages indicates as a rule a mild discrete form. A progressive leucocytosis with an early high polymucocytosis predicts a severe form. The higher the values the more probable the fatal outcome

The early appearance of normoblasts, basophilic stippling and polychromatophilia without evident anemia, is an unfavorable sign. It invariably means the purpuric form of smallpox

Condensation and fragmentation of mature leucocytes are found only in the purpuric form of smallpox. They appear comparatively early in the primary type of purpuric smallpox and are usually accompanied by pathological normoblasts, basophilic stippling and polychromatophilia, without visible anemia

A rapidly progressive, absolute lymphocytosis is a constant characteristic of purpuric smallpox

Scarlatinal and other exanthematous infectious purpura and toxic rash with petechiae etc., can be definitely differentiated from the purpuric form of smallpox during its erythematous stage by these blood findings

THYROID DISEASE Kottman's Reaction, Basal Metabolism and Biologic Tests
Etienne G. Richard, G. Karall E. and Claude F. Compt Rend Soc de Biol
March 19, 1928, XLV 667

A study conducted upon twelve cases in which the results of the Kottman test were correlated with other examinations. The authors believe that the Kottman reaction is unreliable and gives such uncertain results in thyroid disease that it cannot be safely used as a means of diagnosis

WASSERMANN REACTION Wassermann and Flocculation Reaction in Lactetic Milk
Hackman, P. Münch Med Wchnschr May 7 1926 LXIII 774

A report of examinations made in thirty cases

The milk was first shaken with ether, then centrifuged at high speed and tests conducted upon the clear liquid under the fat layer. The author states that the ether extraction did not interfere and that anticomplementary reactions were definitely decreased

Parallel tests were also made upon Berkefeld filtrates of milk

In twenty four nursing women the Wassermann reaction was positive in both blood and milk later becoming negative. Ten were treated in pregnancy, five in childhood, four in pregnancy and childhood

In five cases the blood was negative and the milk positive. In three treated cases both blood and milk were negative

Rosen's observation, that the milk becomes negative sooner than the serum when the patient is treated was not confirmed

Slight oscillations in the strength of the reaction even on the same day were noted. Nonspecific fixation in nonlucetic milk was occasionally encountered.

In luetic milk positive reactions were encountered in as little as 0.025 cc.

The flocculation reactions, (Meenecke's, Dold's, and Sachs-Georgi), were generally variable and much weaker, the first being the easiest to read.

DIAGNOSTIC CASE A Useful Diagnostic Aid Case, Piercy, H. D. Jour. Am. Med. Assn., May 29, 1926, LXXVI, 1689

Piercy describes and illustrates a diagnostic aid case to be carried in the physician's bag.

The case, of German silver, is hinged on one side and pipettes for making blood counts, glass cover slips for blood smears, glass slides for blood or pus smears, a blood agar slant with sterile swab, a sterile test tube for blood or other fluid, hypodermic vials, containing, in one calcium oxybate, and, in the other, sealed capillary tubes for formaldehyde, four 8 cc glass vials containing, respectively, diluting fluid for white and for red cells, alcohol and ether, several needles for venipuncture and lumbar puncture, and a blood lancet.

The cover slips are contained in a small square box in the upper right hand corner. The culture tube made by the Digestive Ferments Company of Detroit, happens to be just the proper size to fit into this case. The spring clips securely sealing the ends of the blood pipettes are of obviously simple construction and should be obtainable from any supply house. The articles are secured in place by nickel plated spring clips of phosphor bronze, soldered to the top and bottom of the case.

LABORATORY TECHNIC

SYPHILIS A Study of Testes from Syphilitic Patients, Saleeby, E. R. Am. Jour. Syph., April, 1926, V, 2

The testes from forty syphilitic human subjects were studied grossly and histologically. In addition to the varying degrees of fibrosis there were, grossly, no characteristic changes. Microscopically, twenty-three cases, or 57.5 per cent, showed pathologic changes commonly attributed to *Spirocheta pallida* infection. In seventeen of the twenty-three cases, the Wassermann reaction was positive, in two, negative, and in four, not made. The Levaditi preparations were negative for the organism in all the cases except in a seven-month-old fetus, where the spirochetes were demonstrated in large number. Eight rabbits were injected intratesticularly, with emulsions from eight cases and observed for four months. None of the rabbits developed syphilomas. The findings in this study tend to show the frequency of the disease in the testicles and the difficulty of demonstrating the organism in the tissues with our present methods and present knowledge of its morphology.

SYPHILIS Twenty-five Years of Congenital Syphilis in Boston, Sylvester, P. H. Jour. Am. Med. Assn., July 31, 1926, LXXVI, 298

The fetal and infantile mortality and morbidity from congenital syphilis should make it a subject of importance in our work.

The Wassermann reaction, the development of arsenical therapy, the specially organized clinic and the social service have combined to effect a satisfactory reduction in mortality and morbidity, provided treatment is instituted during the critical period of from five weeks to two months.

The foregoing factors have had very little effect on cases appearing for treatment much before or much after this period.

More effective treatment for the very early case must be developed, or its prevention must be obtained through treatment of pregnant mothers.

Sulpharsphenamine, intramuscularly, appears to be nearly as effective as neoarsphenamine, intravenously, in causing disappearance of lesions and serologic reversals in early cases.

It appears to be as effective in causing the lesions to disappear in late cases and more effective in reversing the serum

It is possible that a definite reduction in the incidence of congenital syphilis may be brought about through educational and legal measures

ENCEPHALITIS Studies on the Etiology of Epidemic Encephalitis Evans A C and Freeman, W Pub Health Rep, June 1926 xli, No 23 p 1095

A pleomorphic streptococcus, highly virulent for rabbits when inoculated intracerebrally was obtained from the nasal washings, heart blood, and mesencephalon of a case of epidemic encephalitis

In so far as the comparative tests have been made this streptococcus agreed with the streptococci obtained from cases of epidemic encephalitis by Von Wiesner and by Rosenow. Apparently several other investigators have cultivated the same organism in their studies of the disease

When inoculated intravenously into rabbits the streptococcus shows a tendency to elicit localization in the brain

In rabbits and in monkeys it produces nervous symptoms which in some cases simulate the disease in man

Rabbits inoculated with this streptococcus show no inflammatory lesions outside of the central nervous system. The meninges are heavily infiltrated with lymphocytes and leucocytes the inflammation spreads to the cerebral substance by direct extension and along the small vessels penetrating into the brain. There are severe parenchymatous degenerative changes in the nervous tissue and reaction of the neuroglia. The sheaths of the blood vessels are found infiltrated by lymphocytes. The reaction is sometimes most marked in the mesencephalon

In monkeys there is noted a greater tendency toward leucocytic reaction and in two instances large areas of hemorrhagic inflammation in the basal ganglia were noted

POLIOMYELITIS A Skin Reaction In Poliomyelitis Rosenow E C Jour Infect Dis, June, 1926, xxxviii No 6 p 5-9

Rosenow injected 0.1 cc of a 1:100 dilution of a killed culture of a pleomorphic streptococcus

The absence of marked reactions in persons fully recovered from poliomyelitis and who are known to be immune the incidence of positive reactions inversely according to age, corresponding in general to the age incidence of poliomyelitis the strongly positive reactions during the acute stage of the disease and the negative reaction during convalescence, are considered as presumptive evidence that the test is a measure of susceptibility to poliomyelitis

Numerous questions regarding the nature of the reaction have not yet been worked out. The immune serum prepared from horses with the pleomorphic streptococcus, and used with apparent benefit in the treatment of the early stages of poliomyelitis, however has a marked neutralizing power over the toxin, as determined by the skin reaction

COLDS BACTERIOLOGY OF Observations of the Normal Bacterial Flora of Nose and Throat with Variations Occurring During Colds Shibley G S Hanger F M and Dochez A R Jour Exper Med March 1926, xliii No 3 p 415

The normal bacterial flora of the nose and throat of thirteen individuals has been studied over periods ranging from five to nine months

Observations have been made of qualitative and quantitative changes in the flora occurring in the course of colds and of throat infections appearing in the group

The normal basic nasal flora includes *Staphylococcus albus* diphtheroids, and for certain individuals *Staphylococcus aureus* and streptococci. Occasional transient bacteria are Gram negative cocci and nonhemolytic streptococci

The normal basic throat flora includes Gram negative cocci nonhemolytic streptococci,

and for certain individuals "large Gram positive cocci," B influenzae, Bacillus "X," and diphtheroids. Transient organisms are Staphylococcus albus, hemolytic streptococci, Staphylococcus aureus and citreus, and pneumococci.

No bacteria were found in early cold cultures to which a causative role could be assigned.

In the course of colds the basic flora of the nose was often scanty in the early stages. The throat showed reduction of prominence or alterations in predominance of the basic flora.

Certain organisms were prominent in colds, usually, as late or secondary invaders, these included Staphylococcus aureus, hemolytic streptococcus, and B influenzae.

There was a striking increase in the incidence of hemolytic streptococci in throat infections.

WASSERMANN REACTION Concerning the Reactivation of the Wassermann Reaction, Pinard, M. Bull. soc. med. do hop. Paris, May 13, 1926, 141, 724.

Pinard cites a case illustrating the fact that a latent syphilis with negative serology may become positive after a reactivation and reiterates the varied factors which may be responsible for such reactivation such as protein shock or trauma, pregnancy, acute infections, or acute exacerbations of a chronic disease.

He emphasizes that properly conducted tests are highly reliable.

POLIOMYELITIS Further Studies of the Poliomyelitis Precipitin Reaction, Rosenow, E. C. Jour. Infect. Dis., June, 1926, LXXVIII, No. 6, p. 532.

The technique was essentially the same as that used in 1924. It was made as uniform as possible throughout the study. The swabbings were made from the nasopharynx in the same manner, gross contamination from the tongue being avoided. Readings were made under the same conditions of illumination in a darkened room, and in order not to be biased in recording findings, were often made without knowing at the time, the source of the extracts. In many instances tests on duplicate swabbings were made at the same time and repeat swabbings at short intervals. At least two different preparations of the poliomyelitis antistreptococcus serums and four control serums were used throughout the study. In some instances the precipitating power of the serum of convalescent human beings and monkeys was also tested. Blood agar platings of suspensions of the swabbings were made in many instances, both during and following the epidemic.

The results of the precipitin reaction with immune horse serums and extracts of nasopharyngeal swabbings in community and institutional outbreaks of poliomyelitis, proved positive in nearly all frank and abortive cases at the time of the attack, in a high percentage of normal contacts and in persons not exposed to the disease at the time when cases occurred. It proved negative in nearly all of the cases in from two to three weeks after the acute attack had subsided and in normal persons soon after the epidemic had disappeared. In one epidemic, the incidence of positive reactions generally was found low shortly before the occurrence of the first case, high during the period of the epidemic and again low after the epidemic had subsided. The increase in positive reactions as cases of poliomyelitis developed and the decrease as poliomyelitis disappeared occurred rapidly and seemingly independently of exposure to the disease, in isolated households in the country as well as in the urban populations. Persons who were negative to the precipitin reaction on entrance into the epidemic zone soon became positive and reactions resembling abortive attacks of poliomyelitis were common in children. The number of positive reactions in persons who came to the Mayo Clinic from widely separated communities was relatively high during the latter part of August when poliomyelitis was generally prevalent and much lower during the latter part of October after poliomyelitis had largely disappeared. After the epidemic in Rochester had subsided and the precipitin reaction in the population had become largely negative, cases occurred south of Rochester where the number of a positive precipitin reaction was high.

In certain instances polymyositis occurred without exposure within from five to twelve days after the presence of the streptococcus was demonstrated in the throat. Repeated swabbings showed that the carrier state lasts usually from one to three weeks in normal persons. Immunity to polymyositis and the occurrence of the organism in the throat did not run parallel. The positive reactions during epidemics in adults, who are relatively immune, and in children, who are relatively susceptible, were found nearly equally high, and persons who had had polymyositis became carriers of the streptococcus during epidemics quite like persons who had not had the disease.

LEPROSY Notes on the Pathology of Leprosy Wade W. H. Jour. Philippine Islands Med. Assn., Feb. 1926, vi, 37

Wade thus summarizes the views of the Culem leper laboratory.

The lepra cell predominates in the typical leproma but the bacilli are also to be found scattered and massed in tissue spaces and in capillaries. Some of these free lying bacillary masses may have originated in cells which have died and disappeared.

Other leucocytes occupy no essential part of the cytologic picture of leprosy, though lymphoid cells are often present.

No particular study of the blood count seems to have been made but there seems to be a tendency to higher lymphocyte percentages and to exaggeration of leucocytosis in complicating infections.

Anemia is common in advanced cases and becomes marked in protracted lepra reaction.

Lepra bacilli have not as yet been found in the sputum in numbers sufficient to suggest pulmonary involvement when found they apparently come from the upper respiratory passage.

The lepra bacillus is extremely refractory to cultivation or to transplantation to experimental animals.

No specific immunologic test has as yet been found. In the ordinary phases of leprosy, with a carefully adjusted technic, positive Wassermann reactions are not obtained unless there is complicating syphilis or yaws. A certain number of lepra reactions may give weakly positive results, however.

Lepra serums give positive reactions with various flocculation tests.

The red cell sedimentation test is frequently markedly positive.

Determinations of the albumin globulin ratio by viscometry and refractometry indicate that there is often a marked globulin increase.

CEREBROSPINAL FLUID Modifications in the Cerebrospinal Fluid in the Course of Serum Reactions de Lavergne V. and Abel E. B. et M. Soc. Med. Hosp., Paris, March 19, 1926, 1:488

Spinal fluid examinations in twenty subjects presenting an urticaria following the injection of immune serum indicated that the fluid is not entirely normal during the course of serum reactions.

The change, though slight is distinct and evidenced by hyperglycorrhachia, lymphocytosis and the presence of some polynuclears and sometimes merely by a hypertension. Albumin is not increased.

At the beginning of the eruption there is a normal or subnormal cytology followed by the changes above noted, the fluid again returning to normal at the end of the eruption.

The changes correspond to certain clinical symptoms such as headache etc., which may be interpreted as the expression of a moderate meningeal reaction resulting from sympathetic disequilibrium.

SYPHILIS The Vernes Flocculation Test for Syphilis Baylis A. B. Sheplar A. E. and MacNeal W. J. Arch. Dermat. and Syph. August 1925 xii 242

A minute, detailed account of the preparation of the reagent, "perethanol" and of the technic of the test is given in this paper which should be consulted for details too lengthy for abstraction.

In this flocculation test, reported in terms of flocculation in hundredths of milligrams per cubic centimeter, 98 per cent of nongonorrheal give readings of 0.02 mg or less.

A series of 1,000 comparative Vernes and Wassermann tests are reported from which the following conclusions are formulated:

The authors are not inclined to agree with Vernes in discarding the Wassermann test. Certainly, in the United States, the highly sensitive antigen of Kolmer and the use of low temperature for the preliminary stage of the reaction have added to the precision of this test so that it must now be regarded as highly specific and indispensable in the diagnosis and treatment of syphilis. On the other hand, the results of this test require confirmation by other evidence, especially clinical evidence. The serologic test of Vernes presents an additional laboratory check.

The Vernes test is of special value because it avoids the hemolytic system altogether, because its results are read in numerical values directly, and because it frequently conflicts with the Wassermann results in latent and treated syphilis, thus placing the physician on his guard against too slavish acceptance of either serologic result.

When employed to test successive specimens from the same patient, in accordance with the directions of Vernes, this flocculation test is able to give more precise information concerning pathologic variations in the blood than can be obtained by other methods.

The Vernes flocculation test should be regularly employed in conjunction with the Wassermann test as a help in diagnosis and treatment of syphilis.

The result of a single Vernes test is only suggestive unless the reading is high, 0.02 or above.

Repeated tests at definite intervals giving essentially the same reading in spite of provocative treatment speak against a diagnosis of syphilis, even though the reading itself be high.

On the other hand, a relatively low reading which changes appreciably on repeated tests speaks for positive diagnosis.

Conflicts between the results of the Wassermann and of the Vernes tests are especially valuable, as at once a more complete review of all the evidence and the serologic examination of additional specimens is demanded.

MERCURIALS Mercurials A Proposed Method of Laboratory Evaluation and Classification, Peterson, J. B. Jour. Am. Med. Assn., July 24, 1926, LVIII, 223

Briefly the method consists in determining the smallest quantity of drug that will prevent the formation of gas in a yeast sugar mixture of definite strength during a period of one hour.

The actual test solutions were made by mixing 2 cc of a 50 per cent sucrose solution with the desired amount of the drug and sufficient water to make the volume 8 cc. Finally, a 2 cc portion of 20 per cent yeast suspension (Fleischmann's Yeast being used) was added, the whole was shaken and poured into a test tube 10 cm long and 1 cm in diameter. A test tube 15 cm long and 2 cm in diameter was slipped over the open end of the smaller tube and the whole quickly inverted. The linear distance from the end of the smaller tube to the surface of the liquid was carefully measured. After the tube had been kept at exactly 38° C for one hour, this distance was remeasured. The tests of quantitative importance were those containing the smallest quantity of drug that yielded practically no carbon dioxide.

GLYCOSURIA A New Table for Lactose (Milk or Urine) and Glucose (Blood or Urine) Calculation, with Notes on Their Estimation, Haskins, H. D. Am. Jour. Med. Sc., August, 1926, CLXXII, No. 2, p. 256

The author has previously described (JOUR. LAB. AND CLIN. MED., 1923, VIII, 747) a simplified Shaffer-Hartman method which, in the present paper, is again described at great length and applied to the determination of lactose in milk and urine as well as to glucose in blood or urine.

An extensive table is appended from which readings may be made directly without calculation

The paper cannot be abstracted without almost total transcription and should be consulted in the original

CEREBROSPINAL FLUID Studies on the Quantitative Estimation of the Total Protein Content in Cerebrospinal Fluid Young G. A. and Bennett A. E. *Am Jour Med. Sc.*, August, 1926, *clxxii*, No 2 p 249

The following method has been used in over 600 determinations by the authors and by other workers also with perfect satisfaction

Two cc cerebrospinal fluid are measured into an ordinary graduated centrifuge tube ethyl alcohol, 95 per cent, is added up to 8 cc (1 cc of fluid and up to 4 cc with alcohol may be used and gives a better reading where the protein content is greatly increased) The contents are then acidulated with a drop of 10 per cent acetic acid or just a trace of glacial acetic acid The contents are then heated carefully to boiling over a Bunsen burner The protein immediately flocculates. The contents of the centrifuge tube are transferred to a vaccine tube with the capillary tip graduated in 0.01, 0.02, 0.03, 0.04 and 0.05 cc and centrifuged until the precipitate is all thrown down into the capillary tip Some of the precipitate may collect on the sides, then the supernatant solution is stirred with a glass rod and the contents centrifuged again

The amount of total protein normally present in 2 cc of cerebrospinal fluid as determined by this method is from 0.005 to 0.015 cc or from 25 to 75 mg per 100 cc We have been using 2 cc of cerebrospinal fluid because this amount gives a large quantity of precipitate and a better volumetric reading unless the protein content is greatly increased then 1 cc is used The method is simple practical and clinically accurate The determination can usually be completed with three minutes when centrifuged at the rate of 3 000 revolutions per minute The reagents are simple and no elaborate apparatus or preparation of standards are required The vaccine tubes can be procured from any supply house making laboratory glassware The precipitate is readily removed from the capillary tip by using a capillary tip pipette with a rubber bulb from a medicine dropper inserted to the large end

0.01 cc or 50 mg per 100 cc	Normal
0.015 cc or 75 mg per 100 cc	High Normal
0.02 cc or 100 mg per 100 cc	
0.03 cc or 150 mg per 100 cc	
0.04 cc or 200 mg per 100 cc	
0.05 cc or 250 mg per 100 cc	

The authors draw the following conclusions from their observations

Normal fluids contain 25 to 75 mg per 100 cc or 0.005 to 0.015 cc in 2 cc

BLOOD STAINING The Influence of the Hydrogen Ion Concentration on Blood Staining Mommson H. *Klin. Wchnschr.* May 7 1926 *xix* 844

The author concludes that the zone which gives good results in an appropriate staining time is between P_H 6.0 and 7.0

For daily use the author recommends the following mixture of a nearly neutral buffering mixture of even parts of primary and secondary phosphate

m/1 phosphoric acid	67
m/1 sodium hydroxide	100
Distilled water - - -	10000

The electrometric examination of a Giemsa solution prepared with this mixture gave P_H 6.93 The author did not choose the medial of the favorable zone he found but the alkaline pole, because in higher P_H the staining time is shorter By preparatory staining in Jenner's or May Grunwald's method the purple tint of the eosinophiles is avoided

The staining technique is the following

The fixation lasts three minutes and the preparatory staining after May Grunwald in the usual way four minutes. For the Giemsa staining one drop of Gruebler solution is taken for 1 cc of phosphate buffer. It lasts five to ten minutes. Distilled water is used for rinsing. In the preparatory staining the author omits the buffering, because he had experienced that it is disadvantageous with P_H 6.93. Furthermore he rinses with distilled water, because the theoretically correct rinsing with the phosphate buffer is practically negligible at P_H 6.93.

RABIES Eliminating a Source of Error in the Laboratory Diagnosis of Rabies, Bor man, E. K. Am Jour Pub Health, May, 1926, xvi, 476

The following method was devised to eliminate red blood cells as sources of possible error.

Dissect out the brain. Make impression and smears from Ammon's horn, cerebellum, and cerebral cortex in the usual manner. The layer of tissue upon each slide should be made as thin as possible, for a thick layer is more easily washed or rubbed away with subsequent treatment.

Place the slides in the following solution

Methyl alcohol (C P)-----	98 cc
Glacial acetic acid-----	2 cc

Allow the slides to stand in this solution for three minutes. Dry quickly over a flame taking care to avoid intense heating. Transfer the slides to a 10 per cent aqueous solution of potassium carbonate and allow them to stand thus for five minutes. Wash in a gentle stream of tap water. Dry by gently blotting them with smooth absorbent paper. Care must be taken not to wash or rub away any of the tissue adhering to the slides.

Flood the slides with the following dry mixture

Methylene blue (saturated aqueous sol)-----	3 drops
Basic fuchsin (saturated alcoholic sol)-----	2 drops
Tap water -----	20 cc

Warm the slides by passing them through a flame once. Allow them to stain for not more than one minute. The staining time will depend upon the purity and solubility of the dyes used.

Wash the slides in a stream of tap water, dry, and examine for Negri bodies.

Any stain which will demonstrate the presence of Negri bodies may be used. The foregoing stain, which is used in many laboratories, has, however, given the most consistent results with this method. This stain should be reddish blue in color, the red dye should not dominate the blue. It must be kept at ice box temperature when not in use. It should never be used after standing for more than twenty-four hours, as it deteriorates rapidly.

The attempt was made to remove the red cells by the use of acetic acid after the slides had been fixed in C P methyl alcohol. The results so obtained were variable.

By employing a mixture of the two substances the complete destruction of the red cells is effected simultaneously with the fixation of the essential parts of the brain tissue.

The use of the acetic acid produces a change in the staining properties of the tissue, chiefly characterized by a diminished affinity for the dyes used. Other acids have this same general effect upon the tissue, so that it may be ascribed to a change in hydrogen ion concentration of the tissue proteins. The carbonate solution is employed to offset this factor by a process of neutralization.

If the large nerve cells are a clear blue and the matrix a dull red, it is an indication of excessive treatment with the acid fixative or of insufficient treatment with the carbonate solution. Negri bodies are difficult to perceive under such conditions as they are stained but faintly.

A slide properly prepared by this method should show the large nerve cells with reddish blue cytoplasm and deep blue nuclei in a matrix of brilliant red. Negri bodies, if present, will then show a characteristic red with typical granular structure. Unstained, hole like areas will be perceived where red blood cells were located.

REVIEWS

Books for Review should be sent to Dr Warren T. Vaughan, Medical Arts Building,
Richmond, Va

*The Newer Knowledge of Nutrition**

THE most comprehensive exposition of our actual scientific knowledge of nutrition and deficiency diseases which the reviewer has as yet read. Food deficiencies and deficiency diseases necessarily assume a prominent part in the discussion for it is from a study of these diseases especially that we have gained greatest knowledge of nutritional needs.

Throughout the work the "practical application aspect" has been kept to the fore.

Clinical Laboratory Procedure†

THIS volume is written primarily for the practicing physician in an effort to facilitate the performance of routine laboratory analyses. To this end the author has described and illustrated many ingenious homemade articles of apparatus which reduce the cost of laboratory work considerably without sacrificing accuracy.

Only the usually accepted routine studies are described in detail. Prominence is given to the interpretation of findings. We note with pleasure that the minutiae of the more highly technical procedures such as the Wassermann reaction, the colloidal gold reaction, procedures which the physician himself will not do, are omitted and that this space is applied to much better advantage in a discussion of the clinical interpretation of these reactions.

The final chapter consists of a list of the most common diseases with the laboratory studies which are indicated in each. This is very brief but should be of distinct service to the busy practitioner.

Blood Chemistry—Colorimetric Methods‡

THE second edition of Dr Stone's volume follows quite closely the lines of the first. The author limits himself strictly to his text. He does not incorporate alternative methods but uses only those which have been more universally accepted throughout the country. Furthermore he only describes the tests for those substances which are of interest to the practical clinician. We note with pleasure that he has elaborated somewhat on the sections devoted to clinical interpretation of results. The volume covers only a small restricted field but thus it does in a very acceptable manner.

The Newer Knowledge of Nutrition. The Use of Foods For the Preservation of Vitality and Health. By E. V. McCollum, Ph.D., Sc.D., and Nina Simmonds, Sc.D. Cloth. Illustrated. Pp. 610. The Macmillan Company, New York.

A Manual of Clinical Laboratory Procedure—For the Use of the General Practitioner. By Robert A. Kilduffe, A.B., A.M., M.D. Cloth. Illustrated. Pp. 257. The C. V. Mosby Company, St. Louis, Mo. 1926.

Blood Chemistry—Colorimetric Methods—for the General Practitioner with Clinical Comments and Dietary Suggestions. By Willard J. Stone, B.Sc., M.D. Cloth. Illustrated. Pp. 176. Price \$3.25. Paul B. Hoeber, Inc., N. Y. 1926.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

He has included a few dietaries in an appendix. We hope that in his next edition he will extend this so that appropriate sample dietaries will be found corresponding to each of the series of laboratory investigations.

*A Manual of Normal Physical Signs**

THIS is a tabloid manual for the use of the tyro in physical diagnosis. Students should find it of considerable assistance as a corollary to didactic instruction.

It should be of even greater use to instructors in elementary physical diagnosis as a framework or guide on which to develop a scheme of instruction. The book cannot serve as a substitute either for textbooks or for collateral reading.

Potter's Therapeutics, Materia Medica and Pharmacy†

THE fourteenth edition of a volume as well known as this scarcely requires an introduction. The make up follows that of past editions but has been brought strictly up to date. Facility in use as a reference volume is favored by the use of thumb indexes.

The work covers the classification and the administration of medicines, a lengthy materia medica with remarks on the physiologic action and therapeutics of the various drugs, discussion on pharmacy and prescription writing, a long chapter on special therapeutics, alphabetically arranged by disease, which should be of value as a reference manual to the physician, and, in the appendices, Latin terminologies, various tables, and summaries of the existing narcotic and prohibition regulations.

*A Manual of Normal Physical Signs. By Wyndham B. Blanton, B.A., M.A., M.D.
Cloth, Pp. 115. The C. V. Mosby Co., St. Louis, Mo., 1926.
†Therapeutics, Materia Medica and Pharmacy. By Samuel O. L. Potter, A.M., M.D.,
M.R.C.P., London. Revised by R. J. F. Scott, M.A., B.C.L., M.D. Cloth, Pp. 972. P. Blakiston's Sons & Co., Philadelphia, Pa., 1926.

The Journal of Laboratory and Clinical Medicine

VOL. XII

St. Louis, Mo. AUGUST 1927

No. 11

Editor in Chief WARREN T. VAUGHAN, M.D.

Richmond Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON M.D.	CINCINNATI
PAUL G. WOOLLEY M.D.	LOS ANGELES
J. J. R. MACLEOD M.B.	TORONTO
W. C. MCCARTY M.D.	ROCHESTER MINN.
GERALD B. WEBB M.D.	COLORADO SPRINGS
VICTOR C. MYERS PH.D.	IOWA CITY
RUSSELL L. HADEN M.D.	ROSEDALE ILL.
JOHN A. KOEHLER M.D.	PHILADELPHIA
ROBERT A. KILGUS M.D.	ATLANTIC CITY N. J.
A. PARKER HITCHENS M.D.	MANILA P. I.
DEAN LEWIS M.D.	BALTIMORE
GEORGE R. HERMANN M.D.	NEW ORLEANS
HAROLD D. CAYLOR M.D.	ROCHESTER MINN.

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo. as Second Class Matter

EDITORIALS

Laboratory Examinations—Not Laboratory Tests

IT IS greatly to be regretted for many reasons that the crowded condition of the present day medical curriculum prevents more than a general outline of many of the things which it attempts to cover and forces into the consideration of the mechanism pathology and diagnosis of disease, a composite picture of the typical case.

It is, perhaps for this reason and because of the impression thus gained, that there is a perceptible degree of confusion as concerns the relation of laboratory examinations to diagnosis.

Too many men, for example are accustomed to regard the Widal agglutination test as a test for the presence or absence of typhoid fever.

It is of course, nothing of the sort but merely a method devised for the demonstration of the presence or absence of agglutinins for the typhoid bacillus and this information has nothing to do with the presence of typhoid fever and is of no diagnostic significance whatsoever until it has been correlated with all the other findings in the case.

It is obvious that the reaction of an individual to disease is a manifestation dependent upon and influenced by two interacting factors (a) the character and degree of the stimulation excited, and (b) the ability of the particular individual to react

The methods of the laboratory are devised to detect or measure the character and the degree of reaction, and *the results always require interpretation!*

When a leucocyte and differential count are made in the presence of a suspected inflammatory process, one is not making a *test* for the presence of appendicitis, for example, but an examination to detect evidences of reaction to an inflammatory process, just as the temperature and pulse are taken as a part of a study for evidences of reaction to pathologic processes. And just as there are no pathognomonic temperatures, so there are, with only occasional exceptions (as in the leucemias), no pathognomonic leucocyte counts

The sole aim and object of laboratory studies in general is to conduct such examinations as shall serve as a source of information to be acquired in no other way. The meaning or interpretation of the findings is elicited only when they are compared, correlated, and evaluated in conjunction with all the other data, historic, clinical, roentgenologic, etc., obtained by all the available means at hand

If laboratory procedures are consistently thought of as methods of examination rather than as tests for various diseases there will be less clinical confusion in their application and interpretation

—R A K

The Journal of Laboratory and Clinical Medicine

WARRIN T. VAUGHAN, MD, Editor in Chief
Medical Arts Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DIANIS E. JACKSON, MD
University of Cincinnati Cincinnati

Physiology

J. J. R. MACLEOD, MD
University of Toronto Toronto

W. C. MCCARTY, MD
University of Minnesota Rochester

Biochemistry

VICTOR C. MYERS, PhD
Western Reserve University Cleveland

Immunology

JOHN A. KOHLER, MD
University of Pennsylvania Philadelphia

Bacteriology

A. PARKER HITCHCOCK, MD
Bureau of Science Manila, P. I.

Internal Medicine

GEORGE R. HUMPHREY, MD
Tulane University New Orleans

Pathology

WILLIAM C. WOOLLEY, MD
Los Angeles

Tuberculosis

GERALD L. WILB, MD
Casper Sanatorium Colorado Springs

HAROLD D. CAYLOR, MD
Mayo Clinic Rochester, Minn.

Experimental Medicine

RUSSELL L. HADEN, MD
University of Kansas Kansas

ROBERT A. KILDEE, MD
Atlantic City, N. J.

Surgery

DIAN L. WIS, MD
Johns Hopkins University Baltimore

Official Organ of the American Society of Clinical Pathologists

PUBLISHED BY THE C. V. MOSBY COMPANY, 322, PINE BLVD. ST. LOUIS
Entered at the Post Office at St. Louis, Mo. as Second Class Matter

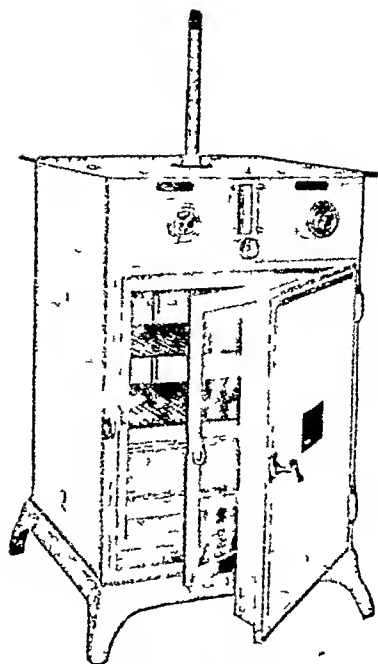
The New Fifth Revised Edition of a Popular Book Physiology and Biochemistry in Modern Medicine

Over 1100 pages 6 1/2 x 9 1/2, with 200 illustrations, including half tones and line drawings and 9 color plates Price, silk cloth binding \$11.00

BY J. J. R. MACLEOD, M.B., LL.D., D.Sc., F.R.S.

Professor of Physiology in the University of Toronto. Formerly Professor of Physiology in the Western Reserve University

Assisted by Roy G. Pearce, L.C. Redfield, W.B. Taylor, J.M.D. Olmsted and by others.



Freas Incubator No 26

Your Incubator Must be Accurate

Just as with your laboratory worker your incubator must be capable of performing the assigned tasks accurately

Accuracy in an incubator is its ability to maintain the temperature constant continuously

This is why the Freas Incubators are used in the leading laboratories

The Thermo Electric Instrument Co.
1215 South Grove Street, Irvington, N. J.

FREAS

ELECTRIC INCUBATORS

The Journal of Laboratory and Clinical Medicine

VOL. XII

St. Louis, Mo. SEPTEMBER 1927

No. 12

CLINICAL AND EXPERIMENTAL ALLERGY

STUDIES IN HYPERSENSITIVENESS

XXIX. ON THE INFLUENCE OF HEREDITY IN ATOPY*

By ARTHUR F. COLE† New York City

I WANT to preface my remarks with a very brief review of the salient facts concerning atopic hypersensitiveness which may be considered established and with which most of you are well acquainted.

1 Atopy (comprising provisionally bronchial asthma and hay fever) is an inherited affection as has been shown by Dr. Cooke and his associates, and confirmed by June Adkinson.

2 In the blood of atopic individual exhibiting a specific cutaneous reaction, sensitizing bodies specifically related to the excitant are nearly always demonstrable. These sensitizing bodies have been called atopic reagents a designation that distinguishes them from the anaphylactic antibodies.

3 Both anaphylactic antibodies and atopic reagents can be produced by human beings conceivably by the same individual.

4 A human individual that produces only anaphylactic antibodies to an antigen is not atopically sensitive to that antigen.

5 The atopic reagents are incapable of conferring anaphylactic hypersensitiveness on the classical test animal (the guinea pig).

Almost every aspect of the subject of human hypersensitiveness presents a question for which no answer has been found and one of the first of these is what shall be included in the atopic group and in this group be sharply defined or not?

Abstracted from the *Proceedings* for the American Association for the Study of Allergy, Washington, D. C., May 16, 19.

†From the Department of Bacteriology and Immunology, Division of Immunology, in Cornell University Medical College and the New York Hospital.

The chief criterion of an atopic hypersensitiveness has been its dependence upon the hereditary influence. Under this criterion, atopy obviously comprises bronchial asthma and hay fever. To these may possibly be added certain forms of drug and food idiosyncrasy, but this question evidently calls for more study. It must not be forgotten that not all forms of natural human hypersensitiveness are subject to the atopic hereditary factor: for example, ordinary serum disease and dermatitis venenata.

A second criterion of atopy was thought, at first, to have been found in the atopic reagins demonstrable with the technic of local passive transfer, but since Rackemann has shown that the serum of worm-infested individuals is capable of passively sensitizing normal skin to worm extracts, this property of atopic serum cannot be used for classificatory purpose. Rackemann's observations have been amply confirmed by Walzer's associate, M. Binnet.

The atopic group is thus left with only one clear bond of union, namely, the bond of heredity.

The demonstration of the familial nature of atopy was made by Cooke and his associates upon two lines of evidence. These writers showed, first, that the percentage of atopic children was greatest (69.5) in families subject to a bilateral hereditary influence, and least (41.1) in those in which the family history was negative for atopic conditions. They also showed that the age of onset of the atopic symptoms is distinctly affected by inheritance. This is seen in the fact that under a bilateral familial influence 72 per cent of the affected offspring begin to exhibit symptoms before the tenth year of age, whereas only about 35 per cent of the affected children, that are subject to a unilateral familial influence, have begun to show symptoms by that age; this percentage falls to 20 among those children whose atopic family history is negative.

The consequences of this evidence seem not to have been fully recognized. The first of these is the simple corollary that, under conditions that permit adequate contact with the excitant, the date of onset of atopic symptoms is determined, in some way, by hereditary influence *for each atopic individual*. In other words, the date at which an atopic individual will begin to experience atopic symptoms is usually predetermined in the inheritance. This principle is in harmony with the well-known fact that many atopic persons have been in constant or annual contact with the excitant (pollens, animal danders) for years previous to the onset of symptoms.

To the question as to the way in which the establishment of atopic hypersensitiveness is influenced by heredity, the first natural suggestion is the one that has been most favored and which is used dogmatically in a recent issue of a semipopular scientific magazine. This suggestion assumes an abnormal permeability of the surface membranes in the hypersensitive individuals. The theory is greatly handicapped with the necessity of admitting that the abnormal permeability in many persons must be exhibited to only one member of a group of similar excitants (a single pollen atopen or a single animal or vegetable protein). Such a specificity of permeability could not be assumed without some experimental evidence.

The studies of Anderson and Schloss, however, and those of Walzer which have revealed an unsuspected normal permeability of the gastrointestinal tract to various common proteins (milk casein, nut, fish, pollen) finally remove the hypothesis from the field.

Anderson and Schloss have shown that the passage of foreign protein through the intestinal wall of normal babies results in the production, by these individuals, of antibodies (precipitating and anaphylactic), yet these individuals exhibited no signs of clinical sensitiveness. Moreover, it is a matter of everyday knowledge that, even the repeated injection of large quantities of foreign protein (horse serum) does not induce atopic hypersensitiveness.

Thus it is seen on the one hand that atopy is not controlled through hereditary differences in permeability of surface membranes and, on the other hand that in the absence of the hereditary factor contact with atopic excitants, parenteral and prolonged is not a sufficient cause of atopy.

When the atopic reagins were discovered and the reaginogenic organ was recognized as distinct in the human being from that which produces precipitin and anaphylactic antibody we thought that this function was, of course, strictly subject to atopic inheritance. In other words, we supposed that the reaginogenic organ functions only in atopic individuals. This seemed indicated by the specificity of the reagins and their constant presence in the blood of asthmatics and hay fever subjects presenting a positive skin reaction. But, as we have said, Rackemann has shown that the serum of worm infested individuals is capable of passively sensitizing the normal human skin to worm extracts, and this observation has since been amply confirmed by M. Brunner.

Rackemann's findings seem to show that the reaginogenic organ is not confined to atopic individuals; it seems to be present in all individuals and responds specifically to substances in worms.

When, however, the reaginogenic organ of the *nonatopic* person is stimulated by the worms to produce reagins, the constitutional hypersensitiveness of atopy seems not to be thereby induced. This is shown by observations of Walzer and Brunner which these experimenters have permitted me to mention in advance of their formal publication. Three of the worm sensitive persons were subject to asthma due to some excitant other than the worms which were not present at the time in the intestinal tract and the injection of a certain quantity of worm extract into these three persons caused a constitutional reaction. Similar injections in worm sensitive but nonatopic persons on the contrary caused no symptoms.

While admitting the probable identity of the antiworm reagins of Rackemann with the atopic reagins one must not overlook the fact that the production of the e bodies against typical atopogens has not been demonstrated in nonatopic persons.

In several papers in which we have discussed the atopic reagins we have emphasized the superior importance of the tissue factor—that is the shock organ in atopy. The determining importance of the shock organ is evident in the well known fact that although reagins are present in the blood in both asthma and hay fever exhibited to the same atopen one individual may

susceptible to Russian thistle pollen. The grass pollens remained potent a much longer time and the results with these extracts in treatment were quite satisfactory.

About the first of August, 1923, we prepared our first glycerine sodium chloride extract and a number of cases who were obtaining poor results with the alcohol salt extract were treated with this new extract. About 85 per cent of satisfactory results were obtained in this small series of cases for the remainder of this season, which continued for another six weeks.

As a result of this experience with the nonglycerinized solution, it was then desirable to determine what percentage of both glycerine and sodium chloride was required to produce a pollen antigen of the highest degree of potency and yet retain its keeping qualities. The solution as recommended by Clock containing $66\frac{2}{3}$ per cent of glycerine and $33\frac{1}{3}$ per cent of saturated solution of sodium chloride was selected as the basic solution. From this solution nine dilutions were made, the weakest one containing one part of Clock's solution and nine parts of distilled water, which gave a solution containing 6.6 per cent of glycerine and 3.3 per cent of saturated solution of sodium chloride, which represented 1.1 per cent of sodium chloride in the total volume. The amount of Clock's solution used in the intermediate solutions was progressively increased by 10 per cent in each dilution. Into these solutions was placed 1 per cent of Russian thistle pollen and extraction was carried out for a period of seven days at room temperature. For convenience we have designated these solutions as Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 as in Table I, solution No. 10 being Clock's or the basic solution and No. 1 the solution containing the smallest amount of Clock's solution.

Skin tests were made with these 10 solutions at the same time upon the same individuals who were known to be sensitive to Russian thistle pollen. The tests were repeated at six-day intervals for a period of two months. In comparing the result of these tests, it was found that solutions Nos. 5 and 6, which contained 3.3 per cent and 3.9 per cent of glycerine and 5 per cent and 6 per cent of sodium chloride respectively, gave larger reactions than the solutions with either higher or lower concentrations of glycerine and salt. At the end of the two-month period all solutions with a glycerine concentration below 4.0 per cent contained bacterial growth and those with 2.0 per cent of glycerine and less gave entirely negative skin reactions. Although solutions

TABLE I

SOLUTION	GLYCERIN PER CENT	SODIUM CHLORIDE PER CENT	REACTION AT ONE WEEK	REACTION AT TWO MONTHS	REMARKS
No. 1	6.6	1	20 mm	0	Bacterial Growth
No. 2	12.2	2	20 mm	0	Bacterial Growth
No. 3	20	3	20 mm	0	Bacterial Growth
No. 4	26	4	23 mm	15 mm	Bacterial Growth
No. 5	33	5	25 mm	25 mm	Bacterial Growth
No. 6	40	6	25 mm	25 mm	Bacterial Growth
No. 7	46	7	23 mm	23 mm	Sterile
No. 8	53	8	18 mm	18 mm	Sterile
No. 9	59	9	15 mm	15 mm	Sterile
No. 10	66	11	10 mm	10 mm	Sterile

Nos 5 and 6 contained a slight bacterial growth, they still gave as strong a reaction as at first. Solution No 7 containing 46 per cent glycerine and 7 per cent of sodium chloride gave reactions almost as strong as Nos 5 and 6 and this solution at the end of three and one half years remains free of bacterial growth and still gives skin reactions as large as those obtained with freshly prepared extracts.

As the buffered glycerimized solutions have been recommended for extracting pollens a study was undertaken to determine the comparative skin reactions to the glycerimized sodium chloride and the glycerimized buffered solutions. As a result of the experiments just mentioned the following solutions were prepared and are referred to by their corresponding numbers.

Solution No 1	Saturated sodium chloride	33	per cent
	Glycerine	66	per cent
Solution No 2	Saturated sodium chloride	33	per cent
	Glycerine	46	per cent
	Distilled water	21	per cent
Solution No 3	Glycerine	66	per cent
	Sodium chloride	7	per cent
	Distilled water	27	per cent
Solution No 4	Glycerine	46	per cent
	Sodium chloride	7	per cent
	Distilled water	47	per cent
Solution No 5	Glycerine	66	per cent
	Sodium chloride	7	per cent
	Sodium bicarbonate	0.27	per cent
	Distilled water	26.3	per cent
Solution No 6	Glycerine	46	per cent
	Sodium chloride	7	per cent
	Sodium bicarbonate	0.27	per cent
	Distilled water	46.75	per cent
Solution No 7	Coccal solution	33	per cent
	Glycerine	66	per cent

To 100 cc of each of these solutions was added one gram of Russian thistle pollen, and extraction was carried out for seven days at room temperature. The solutions were then filtered through ordinary filter paper and the filtered extracts were used for making skin tests. Similar extracts were also prepared from timothy pollen. The Russian thistle and timothy pollens were chosen as they represent the most common pollen offenders in Eastern Washington. Comparative skin tests were made on 100 individuals who were known to be sensitive to the Russian thistle pollen and 50 individuals who were known to be sensitive to timothy pollen. These tests were made by applying successively on the back of each individual, all of the above pollen solutions. Readings were made at the end of thirty minutes and measured in terms of millimeters.

RESULTS WITH EXTRACTS OF RUSSIAN THISTLE POLLEN

The original glycerine sodium chloride mixture as recommended by Clock and designated as Solution No 1 was used as the basic solution, from which all modifications were made. In solution No 2, the percentage of glycerine is

decreased and the sodium chloride content is kept constant. A comparison of this solution with solution No 1 shows that it gives 46 per cent larger skin reactions than did solution No 1. With solution No 3, in which the glycerine content is kept constant and the sodium chloride content is decreased to 7 per cent, there are 58 per cent of larger reactions than with solution No 1. When both the glycerine and sodium chloride contents are decreased as in No 4 solution, however, we find that we have 70 per cent of larger reactions than those obtained with No 1 solution.

Thus, to briefly summarize, 46 per cent of larger skin reactions were obtained where the percentage of glycerine alone is decreased and 58 per cent of larger reactions are obtained where the sodium chloride content alone is decreased. But where both the glycerine and sodium chloride contents are decreased, the larger reactions are increased to 70 per cent.

Since the use of glycerimized buffered solutions have been recommended, we wished to determine whether the addition of sodium bicarbonate influences the extractive properties. Therefore, 0.27 per cent of sodium bicarbonate was added to solutions Nos 3 and 4 and these solutions are designated Nos 5 and 6. In comparing the unbuffered solution No 3 with the buffered solution No 5, we find that with the unbuffered solution 46 per cent of larger reactions are obtained. Further, in comparing solution No 4, the unbuffered solution, with solution No 6, the buffered solution, 56 per cent of larger reactions are obtained with the unbuffered solution. Apparently, then, the addition of sodium bicarbonate adds nothing to the extracting properties of the

TABLE II
REACTIONS TO EXTRACTS OF RUSSIAN THISTLE POLLEN

SOLUTION	NO 1	NO 2	NO 3	NO 4	NO 5	NO 6	NO 7
	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT
Sodium Chloride	(Sat) 33	(Sat) 33	7	7	7	7	0
Glycerine	66	66	66	46	66	46	66
Sodium Bicarbonate	0	0	0	0	0.27	0.27	Cocci's 33
REACTIONS							
Solution No 1 Larger		22	20	14	26	30	22
No 2 Larger	46		30	20	14	44	26
No 3 Larger	58	40		28	46	50	36
No 4 Larger	70	54	50		58	56	50
No 5 Larger	46	36	24	24		30	34
No 6 Larger	42	36	30	18	30		46
No 7 Larger	58	48	38	26	40	36	
No 1 Smaller		46	58	70	46	42	58
No 2 Smaller	22		40	54	36	36	48
No 3 Smaller	20	30		50	24	30	38
No 4 Smaller	14	20	28		24	18	26
No 5 Smaller	26	44	46	58		30	40
No 6 Smaller	30	44	50	56	30		36
No 7 Smaller	22	26	38	50	34	46	
No 1 Same		32	22	16	28	28	20
No 2 Same	32		30	26	20	20	26
No 3 Same	22	30		22	30	20	24
No 4 Same	16	26	22		18	26	26
No 5 Same	28	20	30	18		40	18
No 6 Same	28	20	20	26	40		
No 7 Same	20	26	24	24	26	18	

glycerine sodium chloride mixtures but there is a definite decrease in the extractive qualities of the glycerine sodium chloride solutions when sodium bicarbonate is added

A comparison with Coca's solution was also desirable to demonstrate whether the total replacement of sodium chloride by the bicarbonate solution offered any advantages. In this series it was found that 58 per cent of stronger reactions were obtained with the extract containing Coca's solution compared with the solution recommended by Clock. The solution containing 7 per cent of sodium chloride and 66 per cent glycerine (No 3), however, gave 38 per cent of stronger reactions. The solution containing 7 per cent sodium chloride and 46 per cent glycerine gave 50 per cent of stronger reactions than the extract containing Coca's solution.

TABLE III
REACTIONS TO EXTRACTS OF TIMOTHY POLLEN

SOLUTION	NO 1	NO 2	NO 3	NO 4	NO 5	NO 6	NO 7
	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT
Sodium Chloride	(Sat) 33	(Sat) 33	7	7	7	7	0
Glycerine	66	46	66	46	66	46	66
Sodium Bicarbonate	0	0	0	0	0.27	0.27	Coca's 33
REACTIONS							
Solution No 1 Larger		36	28	8	24	12	36
No 2 Larger	32		24	8	28	20	44
No 3 Larger	48	48		10	36	20	44
No 4 Larger	60	44	84		68	68	92
No 5 Larger	48		48	8		28	2
No 6 Larger	60	64	28	28	64		68
No 7 Larger	36	3	10	4	16	4	
No 1 Smaller		30	48	92	48	60	36
No 2 Smaller	36		48	84	52	64	32
No 3 Smaller	28	24		84	48	68	12
No 4 Smaller	8	8	12		8	28	4
No 5 Smaller	24	28	36	68		64	16
No 6 Smaller	10	20	0	08	28		4
No 7 Smaller	36	44	44	92	2	68	
No 1 Same		32	24	0	28	28	28
No 2 Same	30		28	8	20	16	24
No 3 Same	24	28		4	16	10	44
No 4 Same	0	8	4		24	4	4
No 5 Same	28	20	10	24		8	20
No 6 Same	28	16	10	4	4		28
No 7 Same	28	24	44	4	32	28	

RESULTS WITH EXTRACTS OF TIMOTHY POLLEN

A comparison of Clock's original solution with its modifications shows but little difference between this solution and the solution in which the glycerine content alone has been diminished. When the salt content is decreased to 7 per cent and the glycerine content held constant, the larger reactions are increased to 48 per cent. When both the glycerine and salt are decreased as in solution No 4 the larger reactions are increased to 92 per cent and in this solution we see the largest reactions when compared with all of the other solutions as indicated in Table III.

When the unbuffered solution No 3 is compared with its buffered solution No 5, the greater number of larger reactions are produced by the buffered solution (48 per cent) which would indicate that buffering adds to this solution. Yet the buffering of solution No 4 adds nothing to its extracting properties, since 68 per cent of larger reactions are obtained with the unbuffered solution. When Coca's solution is substituted for the saturated sodium chloride in Clock's solution those solutions containing 7 per cent of NaCl buffered and unbuffered, give a definitely increased number of larger reactions, while in those solutions in which the NaCl content is saturated, the reactions are approximately the same.

DISCUSSION

It must be admitted that the method of comparison by means of the skin test is open to criticism but, since there is no satisfactory method known for standardizing pollen antigens, this method must be considered practical.

From the results of these comparative tests we are led to believe that an unbuffered solution having a sodium chloride content of 7 per cent and give a glycerine content of 46 per cent will produce an antigen that will meet the requirements of at least our section of the country more satisfactorily than any other antigen used in these experiments. In Russian thistle we have an unusually toxic pollen and because of its abundance in most parts of Eastern Washington, Eastern Oregon and Southern Idaho, a very potent extract is essential.

With the above extract we feel that this has been accomplished when the results for the past four seasons are analyzed. During this period treatment was supplied to 2,140 cases, in whom satisfactory relief was obtained in 90 per cent.

CONCLUSIONS

From this data we have concluded

- 1 That the glycerimized extracts of Russian thistle are the only extracts which will retain their potency over a period of months and possibly years.
- 2 By reducing the sodium chloride content to 7 per cent and the glycerine content to 46 per cent, pollen extracts of Russian thistle and timothy are produced which have antigenic properties considerably higher than produced by solutions in which the concentrations of sodium chloride and glycerine are greater.
- 3 The addition of a buffer to this solution does not add to its extracting qualities but, on the other hand, definitely decreases the antigenic properties.
- 4 Satisfactory results in treatment are obtained with extracts containing 7 per cent of sodium chloride and 46 per cent of glycerine.

REFERENCES

- ¹Clock, R O. A Stable Pollen Antigen, *Jour Infect Dis*, 1917, **xxi**, 523
- ²Coca, A F, and Milford, E L. Studies in Specific Hypersensitiveness. **XVII** The Preparation of the Fluid Extracts and Solutions for Use in Diagnosing and Treating Atopic Conditions, *Jour Immunol*, 1925, **v**, No 2, p 555

EOSINOPHILIA IN ASTHMA, HAY FEVER, AND ALLIED CONDITIONS*

By GRAFTON TYLER BROWN, B S, M D, F A C P, WASHINGTON, D C

GENERAL CONSIDERATIONS

EOSINOPHILS are white blood corpuscles of the polymorphonuclear variety, averaging in size from 10 to 14 microns, with definite but pale staining nuclei. Their distinguishing characteristics are the large, coarse, highly refractive granules within the cytoplasm which exhibit special affinity for eosin and other acid dyes. Eosinophiles are quite susceptible to rupture and they frequently are seen in smears as a mass of reddish granules, among which may be found a palely staining lobulated nucleus.

These cells are developed directly from the large eosinophilic myelocytes found commonly in the bone marrow and to some extent, in the spleen. Eosinophilic myelocytes vary considerably in size even under normal conditions, being anywhere from one half to twice as large as the eosinophilic leucocytes. They have rounded nuclei somewhat more heavily staining than the nuclei of the eosinophilic leucocytes and the granules, many times, are not so even and regular as seen in the peripheral blood. The myelocytes appear to be indirectly developed from the myeloblasts going through a transition of the so called premyleocytes in which stage they accumulate their granules and show beginning differentiation into the various varieties of neutrophilic, basophilic and eosinophilic staining affinity.

Under normal conditions the number of eosinophiles found per cubic millimeter of blood ranges from 50 to 400 the normal eosinophilic percentage being about 1 to 4 per cent of all the leucocytes. Usually any increase above 4 per cent up to 10 per cent is considered a moderate eosinophilia from 10 to 20 per cent marked eosinophilia and above 20 per cent, excessive eosinophilia.

The greatest absolute increase in eosinophiles is seen in the early stages of myelogenous leucemia in which a great variety of different forms may be demonstrated not only of the eosinophilic polymorphonuclears themselves, but all varieties of eosinophilic myelocytes. While the total number is increased the percentage is many times not markedly altered, owing to the increase of all the white blood corpuscles of the myelogenic type.

Certain cases of bronchial asthma probably give the largest percentages of eosinophiles noted. Dr. Hunter reported one case to me, in which the eosinophilic count was 87 per cent. The eosinophilia in bronchial asthma is by no means a constant finding. In parasitic infections, particularly the nematodes, eosinophilia is usually a constant finding. This does not hold true, however, of many of the protozoa and some of the cestodes. Eosinophiles in

*Read at the Fifth Annual Meeting of the American Association for the Study of Allergy, Washington, D. C., May 16, 1917.

nematode infections usually range between 10 and 20 per cent, being probably highest in hookworm and trichinella infestations

Eosinophilia is also frequently observed in scarlet fever, which is one of the clinical laboratory aids in differentiating this disease from measles. Eosinophiles are found in increased numbers in the blood in certain skin diseases, particularly pemphigus, prurigo, psoriasis, and urticaria. The presence of eosinophilia seems to depend upon the cause or the nature of the skin disease rather than upon the condition of the skin per se. We also occasionally meet with eosinophilia in subacute and chronic tuberculous conditions, and sometimes in chronic streptococcus infections, and in certain anaphylactic states. Occasionally an increase in eosinophiles is noted in Hodgkin's disease.

Tissue eosinophilia, with or without the occurrence of any material increase of the eosinophiles in the blood stream, is seen in many cases of chronic lymphadenitis, chronic appendicitis, and in a variety of conditions presenting the histologic picture of chronic granulomatosis. Eosinophilia in the blood is also met with in a number of other conditions, in which exhaustive clinical, laboratory, and even autopsy studies fail to reveal the reason for the eosinophilic increase, and for the want of a better understanding, must be termed idiopathic.

The eosinophiles appear to be stimulated in their production by certain substances elaborated by a number of parasites, particularly of the intestinal variety, and by certain tissue changes seen in bronchial asthma, asthmatic bronchitis and other infectious conditions of purely bacterial origin. The eosinophiles appear to be stimulated in their production and attracted to certain tissues of the body in a manner similar to that in which the polymorphonuclear neutrophils are influenced, namely, positive chemotaxis. In the instance of the eosinophiles, however, this stimulation and positive chemotaxis seems to be exerted upon the eosinophilic myelocytes and leucocytes rather than upon the neutrophilic type. The exact nature of this virus or chemotactic influence is not known. Chemical changes in the blood and, so far as can be determined, microchemical changes in the tissues are not of sufficient definiteness to explain the phenomenon.

EOSINOPHILIA IN ALLERGY

In an effort to determine the significance of eosinophilia in allergy,² painstaking analysis was made of 370 consecutive differential leucocyte counts on 346 different patients with asthma, hay fever, or some allied condition.

It is rather generally believed that eosinophilia in asthma or hay fever patients is practically diagnostic of protein sensitization. I am forced to disagree with this opinion, however, as I have seen definitely sensitive asthma or hay fever patients who had no eosinophilia, either during or between attacks. Furthermore, some of the highest eosinophile percentages I have encountered have been in nonsensitive or bacterial cases. There were only four patients in this series with excessive eosinophilia (above 20 per cent), namely, one perennial hay fever patient and three asthmatics, and all four of them were nonsensitive.

In most every patient in this series with marked or excessive eosinophilia the stools were carefully examined for the presence of parasites or ova, but none were found to account for the eosinophilia.

Sex—There were 159 males and 211 females in this series. The average percentage of eosinophiles for the males was about 6 per cent and for the females was about 5½ per cent. Sex therefore has probably no influence on blood eosinophilia in allergy.

Age—The average age of all the patients with blood eosinophilia was about thirty seven years whereas the average age of all the patients whose blood eosinophiles were within normal limits was about thirty nine years. From this it may be inferred that on an average blood eosinophilia is encountered in slightly younger individuals than is a normal percentage of eosinophiles. There was in this series however one patient sixty nine years of age whose eosinophiles were 18 per cent and another patient sixty four years old whose eosinophiles were 22 per cent.

Asthma—By means of skin tests asthma patients are divided into two groups: first, those who are found sensitive to some foreign protein (food, animal epidermal, pollen or miscellaneous irritant) designated as true bronchial or allergic asthma; second, those who are not found sensitive to any foreign protein designated as asthmatic bronchitis or bacterial asthma. In this series, there were 193 differential leucocyte counts on patients with asthma. The eosinophile average for all the sensitive asthma patients was about 7 per cent, and for the nonsensitive asthmatics was also about 7 per cent. Sensitization in asthma, therefore, has no influence on blood eosinophilia.

Bronchitis (Nonasthmatic)—In contrast with asthmatic bronchitis there were 12 cases of nonasthmatic bronchitis in this series with an eosinophile average of only about 2 per cent. In other words, asthmatic bronchitis produces a much higher eosinophile average than does nonasthmatic bronchitis.

Hay Fever—There were 90 differentials on hay fever patients (35 seasonal and 55 perennial) with an eosinophile average of about 6 per cent. The average percentage of eosinophiles for seasonal hay fever was about 5½ per cent, and for perennial hay fever was about 7 per cent, being almost exactly the same in the nonsensitive as in the sensitive patients. In hay fever therefore, just as in asthma sensitization has no influence on eosinophilia.

"Colds"—There were in this series 12 cases of frequent head colds, three of them complicated by sinus trouble. The eosinophile average for this group was only about 3 per cent. It would seem from this that recurrent 'head colds' with or without sinus trouble do not produce as high an eosinophile average as does hay fever.

Eczema—There were 46 differentials on patients with eczema in this series, with an eosinophile average of exactly 5 per cent. The eosinophile average for the nonsensitive eczemas was about 4½ per cent whereas for the sensitive eczemas it was about 6½ per cent. Although it is based on relatively few eczema cases it may be inferred that protein sensitive eczema produces a higher blood eosinophile average than does nonsensitive eczema.

Urticaria—Eleven differentials on patients with urticaria in this series gave an eosinophile average of about 4 per cent. It is evident from these few cases that urticaria does not always produce a definite blood eosinophilia.

Duration of Disease—The average duration of disease of all the patients with blood eosinophilia was about eleven years, whereas the average duration of disease of all the patients whose blood eosinophiles were within normal limits was about thirteen years. From this it may be inferred that, on an average, the longer the duration of disease, the less likely for blood eosinophilia to be encountered. There was in this series, however, one patient with 22 per cent eosinophiles, who had been having asthma for fifty-two years.

Sensitive or Nonsensitive—For the entire series, the eosinophile average of the nonsensitive cases was about $5\frac{1}{2}$ per cent and of the protein sensitive cases was about 6 per cent. It has been previously stated that in asthma and hay fever, protein sensitization has no influence on blood eosinophilia.

Type of Sensitization—In this series, the eosinophile average for the food sensitive patients was about 7 per cent, for the animal epidermal sensitive patients exactly $6\frac{1}{2}$ per cent, and for the pollen sensitive ones about $6\frac{1}{2}$ per cent. Apparently there is no connection between the type of sensitization and the percentage of eosinophiles in the blood.

Sputum—Direct smears from the sputum of 123 patients with asthma or bronchitis in this series, were examined microscopically for the presence of eosinophiles. They were divided into four groups according to the number of eosinophiles present, namely none, few, moderate number, and large number. In comparing the number of eosinophiles in the sputum with the percentage in the blood it was found that in the group with none in the sputum, the blood eosinophile average was about $5\frac{1}{2}$ per cent, in the group with a few eosinophiles in the sputum, the blood average was about $6\frac{1}{2}$ per cent, in the group with a moderate number in the sputum, the blood average was about 8 per cent, and in the group with a large number in the sputum, the blood average was about 9 per cent. In other words, the number of eosinophiles in the sputum of patients with asthma or bronchitis runs directly parallel with the percentage of eosinophiles in the blood.

Blood Calcium—It has been shown in a previous paper¹ that no relation exists between the percentage of eosinophiles in the blood and the calcium content.

All the differential leucocyte counts and stool examinations referred to in this paper were performed by Dr. Oscar B. Hunter, professor of bacteriology and pathology, George Washington University Medical School.

REFERENCE

- ¹Brown, G. T., and Hunter, O. B. Calcium Deficiency in Asthma, Hay Fever and Allied Conditions, *Ann. Clin. Med.*, Oct., 1925, iv, 299.

THE INFLUENCE OF THE CAPILLARY CIRCULATION UPON CERTAIN ALLERGIC CONDITIONS*

By EDWARD SCOTT O'KEEFE, M.D., BOSTON, MASS

THERE is a definite and important relation between the capillary circulation and allergic conditions. Variations in capillary permeability affect the amount and rate of protein absorption from the intestinal tract and later influence the amount of foreign protein which comes into contact with the tissues supplied by the capillary network. The same factors which influence the capillary circulation have long been known to be aggravating factors in allergic diseases.

Within the last few years work by Krogh¹ and later by T. Lewis² have thrown new light upon the functions and structure of the capillaries. The capillaries are no longer considered to be endothelial tubules reacting passively to changes in the heart and great vessels. The Rouget cells have been demonstrated as isolated cells scattered upon the walls of the capillaries. Each cell consists of a mass of nucleated protoplasm with branched processes which encircle the capillary. Upon these cells the capillaries depend for their ability to contract and relax under the influence of certain stimuli.

Both Krogh and Lewis agree that the permeability of the capillaries is increased by a variety of stimuli: mechanical, thermal, chemical and photogenic. Lewis is satisfied to say that the increased permeability arises from these stimuli. He feels that the exact mechanism is unknown. Krogh goes further and states after a variety of ingenious experiments that the increased permeability arises from and is directly due to the dilatation of the capillaries. He feels that there is an actual increase in the size of the interstices of the capillary wall during dilatation which permits at that time, the passage of even large molecules such as the protein molecules into the surrounding tissues.

The conception that increased capillary permeability and capillary dilatation go hand in hand is of great importance in any consideration of allergic conditions. It is significant that the four classes of physical agents mentioned above as stimuli producing capillary dilatation have long been recognized as having an unfavorable influence on eczema. In fact the entire dermatologic program for this disease is in its final analysis, an effort to protect the skin from the reactions following exposure to these agents.

Not only the cutaneous capillaries but those of the intestinal tract play a part in eczema. Faulty digestion resulting in irritating intestinal contents might be expected to cause a change in the permeability of the capillaries of the villi of the small intestine. In this connection the work of Schloss³ and

*Read before the American Association for the Study of Allergy at Washington, D. C. May, 16, 1917.

Urticaria—Eleven differentials on patients with urticaria in this series gave an eosinophile average of about 4 per cent. It is evident from these few cases that urticaria does not always produce a definite blood eosinophilia.

Duration of Disease—The average duration of disease of all the patients with blood eosinophilia was about eleven years, whereas the average duration of disease of all the patients whose blood eosinophiles were within normal limits was about thirteen years. From this it may be inferred that, on an average, the longer the duration of disease, the less likely for blood eosinophilia to be encountered. There was in this series, however, one patient with 22 per cent eosinophiles, who had been having asthma for fifty-two years.

Sensitive or Nonsensitive—For the entire series, the eosinophile average of the nonsensitive cases was about $5\frac{1}{2}$ per cent and of the protein sensitive cases was about 6 per cent. It has been previously stated that in asthma and hay fever, protein sensitization has no influence on blood eosinophilia.

Type of Sensitization—In this series, the eosinophile average for the food sensitive patients was about 7 per cent, for the animal epidermal sensitive patients exactly $6\frac{1}{2}$ per cent, and for the pollen sensitive ones about $6\frac{1}{2}$ per cent. Apparently there is no connection between the type of sensitization and the percentage of eosinophiles in the blood.

Sputum—Direct smears from the sputum of 123 patients with asthma or bronchitis in this series, were examined microscopically for the presence of eosinophiles. They were divided into four groups according to the number of eosinophiles present, namely none, few, moderate number, and large number. In comparing the number of eosinophiles in the sputum with the percentage in the blood it was found that in the group with none in the sputum, the blood eosinophile average was about $5\frac{1}{2}$ per cent, in the group with a few eosinophiles in the sputum, the blood average was about $6\frac{1}{2}$ per cent, in the group with a moderate number in the sputum, the blood average was about 8 per cent, and in the group with a large number in the sputum, the blood average was about 9 per cent. In other words, the number of eosinophiles in the sputum of patients with asthma or bronchitis runs directly parallel with the percentage of eosinophiles in the blood.

Blood Calcium—It has been shown in a previous paper¹ that no relation exists between the percentage of eosinophiles in the blood and the calcium content.

All the differential leucocyte counts and stool examinations referred to in this paper were performed by Dr. Oscar B. Hunter, professor of bacteriology and pathology, George Washington University Medical School.

REFERENCE

- ¹Brown, G. T., and Hunter, O. B. Calcium Deficiency in Asthma, Hay Fever and Allied Conditions, *Ann. Clin. Med.*, Oct., 1925, 17, 299.

ple, if there is a stiff gale throughout the early morning hours, during which time the pollination is the heaviest it would carry more pollen into the air than a similar gale during the evening. The lowest wind velocity over a period extending from August 11 to October 1 for any one day, in Oklahoma City, was six miles and the highest velocity was 17.2 miles. The average wind velocity over the period mentioned was 9.9 miles.

From a clinical standpoint the wind is a very definite factor in the cause of hay fever symptoms for on windy days patients will complain more bitterly. Our pollen counts show that when all other factors are equal, the number of pollens on the plate is almost in direct ratio to the wind velocity. It ought to be this way. For example if the wind is blowing at five miles an

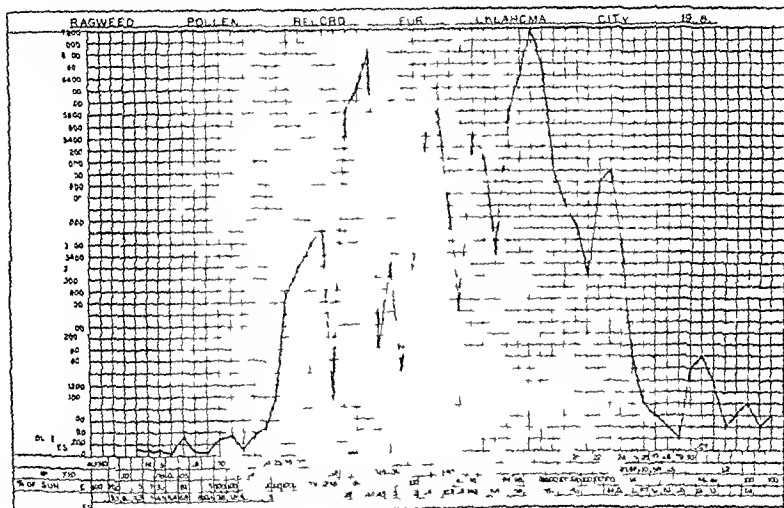


Chart L.—A study of the effect of precipitation and wind velocity on the pollen content of the air.

hour, let us assume five pollen granules would come in contact with the pollen plate or the mucous membrane. If the wind were blowing at fifteen miles an hour, there should come in contact with our pollen plates and with the mucous membrane of the hay fever sufferer three times five pollen granules or fifteen pollen granules over the same surface naturally would produce much more severe ones.

THE POLLEN PLATE BY SUNSHINE

Every botanist who has collected pollen has noticed the poor production of pollen on cloudy days. In fact one may fill the pollen house with pollinating plants and find practically no pollen at all on the following morning if it

is cloudy, while if the sun shines, the production will be very great. From Chart I the effect of sunshine can be readily seen. Over the period in which the percentage of sunshine is 100 or nearing 100, even if the rainfall is fairly great, the pollen content is high, but if the rainfall is zero and the percentage of sunshine is low, the pollen count is relatively low. The figures relative to sunshine shown in the chart are the percentages of possible sunshine during the day. During the period from August 11 to October 1 there were twelve days with 100 per cent sunshine, and eleven other days with more than 85 per cent sunshine. From a clinical standpoint, one soon learns that sunshine plays a very definite part in determining the amount of pollen that gets into

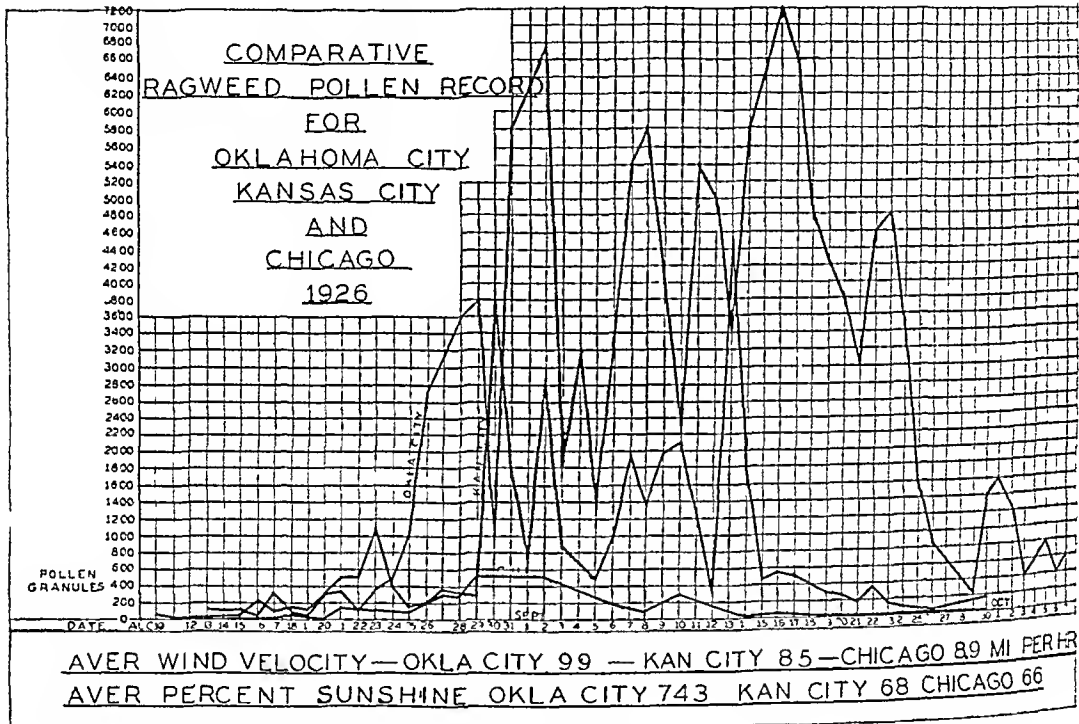


Chart II—A study of the factors which might make a difference in the pollen content of the air in three large cities

the air, and from our study of plant life we learn that the amount of sunshine determines very largely the amount of pollen that is actually liberated from the pollen pods.

IMPORTANCE OF PLANT LIFE

The greatest factor in determining the amount of pollen that can be found in the air is that of the abundance of plant life. If the plant life is scarce, it makes no difference whether the sunshine is 100 per cent, the wind velocity great, or the rainfall light, for it would be impossible for much pollen to be found in the air. The actual location of the majority of plants is not such an important factor if they are within range of three or four miles of a city, for from this distance the pollen will be brought in by means of the

wind. This will hold true for all plants with the exception of a few whose pollen is very heavy. We found it made very little difference where our plates were located as to the relative pollen count.

COMPARATIVE POLLEN RECORD FOR OKLAHOMA CITY, KANSAS CITY AND CHICAGO

During the late summer and fall of 1926 we made daily ragweed pollen counts, which are shown in curve of Chart II. Similar work was done in Kansas City as is shown in the same chart, likewise the record of a pollen curve for Chicago in 1925 is shown. In observing the three curves, one can readily see a vast difference between the pollen content of the air in Chicago and in Kansas City, or between Chicago and Oklahoma City. The difference between the Kansas City and Oklahoma City curves is not quite so striking.

You will note that the highest pollen content of the air in Chicago for 1925 was 480 pollen granules, for Kansas City in 1926 was 4800 granules, and for Oklahoma City 7200 pollen granules. It is also of interest to note that the dates on which the first pollen granules were found in all three cities were practically the same—Aug. 12 and 13—and that the first peak for all three cities was on Aug. 29 and 30. One will notice from the Chicago curve that, from Sept. 14 and on, the curve hugs the base line and that the Kansas City curve drops to a low ebb about that time and continues with a small amount of pollen from then on. The Oklahoma City curve does not drop until Sept. 25.

The question naturally arises as to why the pollen content of the air is so light in Chicago as compared with Oklahoma City and Kansas City. Is it the difference in the wind velocity, the sunshine or precipitation between these three cities, or is there some other factor? If you will note from the chart, the average wind velocity over the period studied in Oklahoma City was 9.9 miles, Kansas City 8.5 miles and Chicago 8.9 miles. You will readily see that the difference is very little. Therefore the difference in wind velocity could not be the main factor. The average per cent of sunshine in Oklahoma City was 74.3, in Kansas City 68 and in Chicago 66. Although there is some difference in the per cent of sunshine in the three cities it does not seem reasonable to believe that the per cent of sunshine could possibly make the difference in the pollen content of the air.

The rainfall in Chicago in 1925 is not shown on this curve but from the meteorologic reports it was not as great as that of Oklahoma City in 1926. So the difference in rainfall of the three cities could not make the difference between the amount of pollen that is actually found in the air. Therefore the factor which has made such a wide range of difference in these three pollen curves must be one other than rainfall, percentage of sunshine, or wind velocity. In my judgment there is only one other factor that it could possibly be and that is the difference in the amount of plant life near these three large centers.

If you will travel from Oklahoma City to Chicago during August and September you will find that the ragweed growth, like the growth of other plants around Oklahoma City, is greater than that of the same plants around Kansas City. The difference is great enough so that it is fairly easily de-

tected As one goes to Chicago, further on he can see a striking difference between the plant life around Oklahoma City and Chicago When one takes into consideration the fact that at least 50 per cent of the time the wind is blowing off the Lakes, thereby coming off a territory in which there are no plants, it seems reasonable to believe that the amount of pollen in the air in Chicago should be at least 50 per cent less than the pollen in the air in Oklahoma City, even if the plant life on the other side of that city were equal to that of Oklahoma City Although the Lakes reduce the amount of plant life that might produce pollen very largely, the pollen content in Chicago is so much less than that in Kansas City and in Oklahoma City, that even taking the Lakes into consideration, it could not reduce the content to such a low ebb unless there were another factor, and that other factor, I am sure, is that of the difference in the abundance of wind-borne pollinated plants around the three centers mentioned

THE ORAL ADMINISTRATION OF POLLEN*

By J H BLACK, M D, DALLAS, TEXAS

THE first definite attempt to immunize pollen-sensitive individuals to their specific pollens was that of Dunbar¹ who, working on the hypothesis that the active substance of pollen was a toxin, injected animals with pollen and used the antiserum thus obtained in an effort to secure a passive immunity in his patients In 1911 Noon² and Freeman,³ with the same belief regarding the active substance, gave a series of injections of pollen extracts to their patients with the hope of producing an active immunity and protection against clinical hay fever

While our present form of treatment dates from the work just mentioned and that of American workers who quickly followed them, our opinions as to the active substance of pollen and our ideas as to the mechanism by which relief is obtained have undergone radical revision Sufficient data are not available to permit definite conclusions about either but one may be permitted to say, first, that evidence is accumulating which tends to confirm the belief that the active constituent of pollen probably is not protein, second, that no one believes that pollen therapy brings about a true immunity, third, that recent evidence discredits the idea that treatment is specific desensitization,⁴ and, fourth that no evidence exists for the assumption that a tolerance is induced The finding of a specific "reagin"⁵ in the blood of pollen sensitive individuals brought some hope that it might explain the situation but the demonstration⁶ that reagin does not in any detectable manner alter the activity of the "atopen," leaves the question unanswered

Since it has been assumed that the atopen in pollen is protein or indissol-

*Read at the Fifth Annual Meeting of the American Association for the Study of Allergy Washington D C May 16 1927

ably associated with it, hypodermic administration has seemed the only rational means of treatment. If this assumption is not correct, the necessity for this method no longer exists and other avenues of approach may be considered. There are at least three objectionable features of hypodermic therapy. First, there is always a certain amount of pain which becomes a matter of consequence with children and those to whom concentrated glycerine saline extracts are given. Second, patients who have no physician accessible must resort to self-medication or be denied treatment. Third, and by far the most important, constitutional reactions occur not infrequently in spite of all precautions.

In order to determine if protection could be secured by the oral administration of pollen, and also to learn something of the absorption, distribution and elimination of pollen thus administered, giant ragweed pollen—to which I am sensitive—was taken orally by me. This is a brief preliminary report upon this work.

The extract used was a 5 per cent glycerine Coca solution of giant ragweed made by standing twenty-four hours at room temperature and filtered through paper. One tenth c.c. of this extract diluted 1:10,000 was the minimal amount required to produce a mild hay fever within two minutes after being instilled into my nostril. A dilution of 1:100,000 injected intradermally into my forearm produced a typical wheal with pseudopods.

The initial dose was 0.1 c.c. of the 5 per cent extract diluted with approximately 200 c.c. of water and taken on an empty stomach with the belief that it would be passed rapidly into the intestine without stimulating gastric secretion. The dose was increased by doubling until 10 c.c. was reached, after which 0.1 c.c. increments were used. Doses were taken three times daily. The maximum dose was 25 c.c. which gave a total of 75 c.c. in the twenty-four hours. This is fifteen times the usual dosage usually required hypodermically in our locality and which has protected me during the past three ragweed seasons. The total amount taken was 34.5 c.c. in seven days' time.

Since this was done outside the ragweed season an attempt was made to measure the degree of protection secured by the lowering of the sensitiveness of the nasal mucosa to instillation of ragweed pollen into the nostril. Blood was drawn from a vein each day, the serum separated and used for intradermal injection to determine the presence of ragweed atopen. Urine was collected, filtered through a Berkefeld filter and tested intradermally to determine if elimination occurred in this way. Bees were extracted with distilled water and put through a Berkefeld candle then used intradermally to learn if some of the material was coming through unabsorbed.

The first statement which can be made regarding our findings is that the quantities of pollen used produced no unpleasant symptoms of any kind. There is little taste to the well diluted extract and nausea or pain was not experienced. No constitutional reaction occurred which is in marked contrast to my experience in taking hypodermic therapy.

Whereas before this treatment 0.1 c.c. of a 1:10,000 dilution of this extract produced sneezing in two minutes when instilled into my nostril (and reaction to this minimal amount is a constant), on the day following cessation

of treatment 0.1 cc of a 1-1250 dilution, or eight times the usual amount, was required. Whether this is an *entirely* reliable index of protection I do not know. It certainly imitates, as near as may be, the natural mode of attack.

Levine and Coca⁷ have injected pollen intravenously in normal individuals and found it in the blood up to the seventh day and demonstrated it in the urine during the first forty-eight hours. Two sensitive individuals who had received pollen treatment over a period of eight weeks showed none in their blood.

My blood was drawn on the last two days of treatment, and one day, five and seven days respectively after treatment was discontinued. The serum was separated and injected intradermally into my forearm, into passively sensitized areas in a normal skin, and into a nonsensitive skin.

In my skin the reactions obtained during the last two days of treatment equalled in size those resulting from the injection of a 1-20,000 extract. One day after treatment was stopped the wheal was slightly more than one half as large, while on the fifth and seventh days after treatment the reaction was not appreciably larger than the control. Injections of serum into a nonsensitive skin were made at the same time as the above and were uniformly negative. In sensitized areas in a normal skin reactions paralleled closely those in my own arm. Evidently the atopic substance was being absorbed in appreciable quantities and was unchanged by contact with reagin. Elimination from the blood was rapid.

Since it has been shown that reagin is neutralized in the skin and *in vitro* by contact with its specific atopen,⁶ it is interesting to note that, in this instance, atopen and reagin apparently existed coincidentally and could be demonstrated in the serum at the same time. One-tenth cc of my serum drawn on each of the last two days of treatment was injected intradermally into a normal individual. On the following day a 1-100 dilution of pollen was injected into these sites. Typical reactions were obtained which were only slightly smaller than those resulting in sites sensitized by my serum drawn prior to treatment. It would seem that, while the atopen was present in the serum, in demonstrable amount, it was not sufficient to neutralize all the reagin, at least.

The filtered urine was tested during the last three days of treatment. The size of the wheal in sensitized skin increased progressively paralleling the increase in amount of pollen ingested, while in normal skin reactions did not occur. Urine passed on the seventh day after treatment gave a small, but definite, reaction.

A watery extract made of feces passed on the last two days of treatment produced large wheals in sensitized skin and no reaction in the normal.

SUMMARY

1. The oral administration of large doses of pollen extract to a sensitive individual is apparently devoid of danger and causes no unpleasant symptoms. It is probable that the slow absorption by this route makes it possible to administer large amounts without danger of constitutional reactions.

2 That an appreciable amount of the pollen is absorbed is shown by its presence in the circulating blood and its elimination in the urine That some of it is not absorbed is shown by its presence in the feces

3 A certain amount of protection may be assumed by the lowered sensitivity of the nasal mucosa Clinical protection could not be proved in this work because it was done outside the pollen season

4 The coexistence of atopy and reagin in the circulating blood is shown

CONCLUSION

While this fragmentary report is not offered as final evidence it would seem justifiable to conclude that the oral administration of pollen extract offers a satisfactory means of securing protection against pollen and is free of the objectionable features of hypodermic therapy

NOTE—Since the above was written approximately one hundred fifty cases of pollen asthma and hay fever have been treated with this method with most gratifying results A detailed report is in preparation

REFERENCES

- ¹Dunbar Munchen 1903
- Neon, L Lancet 1911 i 1372
- ²Freeman J Lancet, 1911, ii, 814 1914, i, 1178
- ³Levine Philip and Coca Arthur F Jour Immunol 1926 ii 449
- ⁴Prausnitz and Kustner, H Centralbl f Bakt Orig 1921, lxxxv, 160
- Coca A F, and Grove E F Jour Immunol 1921, v 449
- Levine Philip and Coca Arthur F Jour Immunol 1926 ii 411
- ⁵Levine Philip, and Coca, Arthur F Jour Immunol 1926 ii 449

CLINICAL INVESTIGATIONS IN ALLERGY

A REVIEW OF 189 PATIENTS OF ASTHMA AND HAY FEVER CLINIC*

BY LEON UNGER, M.D.,† CHICAGO

IN MAY, 1924, a clinic for the care of patients suffering from the so called allergic diseases was opened at Northwestern University Medical School At first there were but few patients but the number has increased steadily to the present time

This group under study consists chiefly of cases of bronchial asthma and hay fever, besides these there are a few cases of allergic rhinitis, urticaria and angioneurotic edema Other diseases such as eczema and epilepsy have for some years been thought by some to have a relation to allergy, but the connection is not as well established as in those just referred to

One hundred and eighty nine patients comprise this study There are numerous others who were examined in the clinic and immediately referred to some other department or who came only once or twice to the clinic

*Read before the American Association for the Study of Allergy Washington D C May 16 1927
 †Assistant Professor Department of Medicine Northwestern University Medical School

These are not counted in the above number. Of these cases 135 are white and 54 colored, 96 males and 93 females. The average age is 32. The average duration of symptoms is six years. There is a family history of asthma or hay fever in 36 of the cases or 19 per cent.

Complete histories and physical examinations were done in all of the cases. In addition all had Wassermann, urine and blood count tests done, a large majority also had sputum and chest x-ray examinations. Most of the patients were also carefully examined in the nose and throat dispensary and where indicated x-rays of the sinuses were carried out. Every effort was made toward correct diagnoses.

Bronchial asthma was diagnosed in 121 of the 189 cases. This diagnosis was based chiefly on the history of the attacks of dyspnea, cough and wheezing starting usually in children or young adults. Physical examinations varied, wheezing sounds and the typical musical chest findings were present during an attack and often between attacks, but frequently there were no râles between spells.

Skin tests were done more or less completely on 88 of these 121 cases. Thoroughness was our slogan and we tested out the patients as completely as possible. Most of these patients had from 100 to 200 different cutaneous tests performed, in addition some intracutaneous experiments were carried out. In the other 33 cases tests were either not done at all or were incomplete.

Of the 88 patients suffering from bronchial asthma who were thoroughly skin tested 74 or 85 per cent gave positive findings, 13 or 15 per cent were completely negative, 21 of the positives were found in patients who had seasonal asthma and hay fever and who only developed asthma during the pollenating season of the weeds to which they were sensitive. All these gave positive skin tests to one or more pollens, especially to giant or short rag weed, the chief causes of hay fever in this region. As a rule the dyspnea in this group occurred about one week after the hay fever symptoms began and some of the cases were acutely ill at this time.

The other 54 positive cases of bronchial asthma gave 126 positive skin tests to different substances. There were 64 positive to animal derivatives, 23 to food substances, 23 to house dust, and 16 to miscellaneous substances. From this number it is at once apparent that most cases are sensitive to more than one substance and this fact emphasizes the necessity of complete testing of patients. It is not advisable to quit trying when one positive is found.

Of the animal materials goose, duck and chicken feathers comprise 25 of the positives, cat hair 8, horse dander 7, cattle hair 5, pigeon feathers and squirrel fur 4 each, canary feathers 3, 2 each of dog hair, camel hair, and rabbit hair, one each of mouse hair and parrot feathers.

The food substances were scattered and not nearly so important, but did include 3 cases each of egg and wheat which are frequent causes of bronchial asthma. The other 17 food positives were divided among the vegetables, chiefly, and were apparently of little importance as causative factors.

The miscellaneous group had 16 positives, chief of which was olive oil, the basis of face and talcum powders—there were 7 positives to this. Py-

rethrum, the main ingredient of insect powders, was second with 4, the other 5 consisted of 1 positive for each of silk, tobacco kapok (silk floss) henna and face cream

There were 23 positive tests for house dust. Dust was collected from several houses and extracted according to the Coca method and injected intracutaneously, using a control of the extracting fluids. Dust is obviously a mixture and as obviously must vary more or less in different homes. Up to the present time we have not had facilities for collecting and extracting dust from each patient's home. This is the ideal we have in mind and will carry out as soon as we have sufficient assistance. We approached this work about a year ago in a very skeptical frame of mind even though some excellent results had been reported from the Cornell clinic. We have had some encouraging results, however, and the work is still going on.

The treatment carried on in the clinic is both symptomatic and specific. The former consists of adrenalin and ephedrin for attacks of asthma, cough mixtures, potassium iodide calcium lactate thyroid etc. The specific treatment aims at elimination of the offending substance, where possible and desensitization where necessary. Pillows are banished in the feather cases, animals in those affected by them, eggs and wheat are withdrawn from the diet where indicated, face powder removed in those cases sensitive toorris root. We have used ephedrin in about 75 cases to date and can say we have had some fairly good results and some not so good. With better grade ephedrin now available we hope for better success.

Desensitization has been attempted where advisable by increasing hypodermic injections of the offending substances—beginning with 0.1 cc, usually, of 1:10,000 or 1:100,000 dilution and working up to 1:500 or in pollens, to 1:100 dilution. Altogether about 20 to 30 injections or more have been given in each case. Dust injections have been given beginning with 0.05 cc and increasing the same amount each time till 1 cc dosage has been reached, then we repeat this dose once or twice weekly.

In certain cases especially in those who give no positive skin tests other measures have recently been tried, including vaccine therapy, mercury quartz vapor lamp and x ray treatments and intravenous injections of sodium iodide and calcium chloride. The work along these lines is too new for definite reports but we feel strongly that antogenous vaccine and quartz lamp treatments offer considerable promise. We hope to report on these aspects at some future time.

The hay fever cases have been given both preseasonal and coseasonal injections of the offending pollens and we have had good results in both groups. We prefer to give treatment before the time of pollination but in those patients who report during the season we have been agreeably surprised at some excellent results from a few injections.

The results of treatment in our asthma and hay fever cases have been as follows:

BRONCHIAL ASTHMA	121 CASES
Good results	12
Improved	26
Not improved	7
Not returned or incomplete	76

Of the patients who showed positive skin tests and who have more or less completed treatment 35 out of 38 showed at least some improvement. Of these 10 were rendered practically free from asthma.

Three of seven asthma patients who were thoroughly tested and found negative were somewhat improved by symptomatic treatment, four cases still have attacks.

HAY FEVER	47 CASES
Good results	14
Improved	11
Not improved	1
Treatment incomplete	21

Thus we find 25 of 26 completed cases more or less improved by pre seasonal or coseasonal pollen injections, we especially noted that those cases with combined asthma and hay fever were most benefited by this treatment and practically every one was rendered free from asthma.

ALLERGIC RHINITIS

This condition is characterized by more or less sneezing and rhinitis occurring all year round. Pollen tests are usually negative here while tests for grass root, feathers or wheat flour are frequently positive. Removal of the cause and desensitization usually give excellent results. We had only four cases of this affection of whom three were sensitive to grass root, and one to wheat. There is no doubt that a large percentage of persons, especially women, who have frequent attacks of rhinitis belong to this sensitive group, and, likewise, if physicians who treat these cases would have them skin tested many more would be unearthed, their offending substance or substances removed, and the patients benefited.

There were five cases of urticaria and angioneurotic edema. Skin tests were negative in one and tests were incomplete or not done in three. One splendid result occurred lately, a woman strongly positive to silk. She has improved remarkably on withdrawal of all silk garments.

Of 189 patients 38 were definitely nonallergic and in most of these skin tests were not carried out. In the few tested no positives were found. Of these 38, 13 were diagnosed cardiorenal cases and gave the the usual history of dyspnea on exertion beginning late in life and associated with abnormal heart or kidney findings. There were six cases of tertiary syphilis, possibly syphilis of the lung, these were most interesting cases and some had severe asthma closely simulating bronchial asthma. Specific luetic treatment was given these patients with some very excellent results.

Three cases were diagnosed pulmonary tuberculosis. This is a small number when we realize that a large percentage of all asthma cases are wrongly diagnosed as tuberculous and many of our cases have been in sanatoriums. From our experience we would suggest that a patient having attacks of cough, wheezing, and dyspnea who has asthmatic type of râles only, and who has had repeated negative sputums, is probably not tuberculous and should be tested for bronchial asthma.

One disagreeable fact stands out in this brief review and that is the large number of cases who have not returned for further testing. Some came only once, others only a few times. This feature is characteristic of all dispensary work and exists to a lesser extent in private practice as well. Patients wander around from clinic to clinic as they often do from one physician to another. We have wasted a good deal of time and strength caring for these cases with no apparent good either to them or to us.

In an effort to lessen this evil we now hand each new case a copy of the following *Instructions to Patients Suffering with Asthma*. Asthma is a chronic ailment and one which causes a good deal of suffering. We know that among the chief causes of asthma are hair and dander from animals, foods, pollens from weeds, bacteria and dust.

"A great deal of time and patience is required to discover the cause in any one particular case and then there follows a long course of treatment which depends on the cause in each case. All this takes time but in order to get good results in this long drawn out sickness *we must have your help*. You must be patient and come regularly when told to do so.

"If you do not intend to go through with the tests and treatment we carry out here, we prefer that you do not begin at all as we do not want to waste your time or ours. The results here have been excellent in most cases, if you cooperate and follow instructions you also have a good chance to obtain relief."

We hope the above pamphlet will help our attendance.

In conclusion I want to thank Drs. S. M. Kienberg and S. I. Taub for their invaluable services both in the clinic and in preparing these statistics. We trust that at some future time we may again have the opportunity to come before you with some newer and more important discoveries.

THE RÔLE OF THE STRUCTURAL FEATURES OF POLLEN GRAINS IN IDENTIFYING THE MOST IMPORTANT HAY FEVER PLANTS OF CALIFORNIA*

By GEORGE PINESS, M D , AND H E McMINN, A B , A M , LOS ANGELES

INTRODUCTION

ATTEMPTS at identifying wind-blown pollens have been made by various workers in the field of allergy. The pollen is secured by exposing microscopic slides covered with a thin layer of glycerin, corn oil, or cottonseed oil. Most of these attempts have been very limited on account of the almost total absence of structural keys for the identification of the pollens found upon the slides.

During the past three years we have made studies of about 350 species of wind-blown pollen plants of California. The pollens from about 180 of these species have been included in the treatment of allergic patients in our laboratories. The structural features of these pollens have been carefully studied, and a key has been prepared which will enable us to determine, as far as possible, the species of plants whose pollens might be found in the atmosphere throughout the state of California.

METHODS

The pollens were examined fresh from the flowers, several days after falling, and after being treated with ether. These three pollen conditions were designated as fresh, dry and ether-dry respectively. Observations were made with the aid of the 4 mm. and oil immersion objectives upon the three pollen conditions, mounted dry, in water and in balsam. Dry and ether dry pollens when mounted dry were similar in all visible characteristics with the exception of color, whereas the fresh pollen mounted dry varied much in shape and in other visible characteristics, this being due perhaps to the amount of desiccation occurring before and during shedding. Water and balsam mounts caused most dry and ether-dry pollen to become spherical, exceptions being noticed in a few species with oval, elliptical and pyramidal pollen grains. These simply swelled slightly, retaining their characteristic shape when dry.

Water mounts proved the most valuable in studying the germinal pores and certain features of the exine, while dry mounts were used for the study of size, shape and furrows caused by desiccation. We have considered the dry condition the natural condition of pollen for our studies because the pollen, when wind-blown, soon becomes desiccated and shrunken. This is in agreement with the studies of Pope.⁴

Photomicrographs were made of all pollens and these aided materially in supplementing the microscopic observations.

*Read before the American Association for the Study of Allergy, Washington D C
May 17, 1927

RESULTS

Although most of our results are embodied in the key which follows, it seems important to call attention to a few outstanding observations and conclusions. The grass family Gramineae contains more possible hay fever species than any other plant family. Over thirty genera, including about seventy species, of grass pollens were studied. Dry mounts of dry pollen revealed that all species of grasses contained many pollen grains resembling in shape and appearance grains of half ripe field corn, i.e. pyramidal with shrunken cavities on their surfaces. This character while not uniformly present in all pollen grains serves as one of the best aids in identifying grass pollens. Without a single exception all the species showed a single germinal pore and the presence of abundant starch. These characteristics showed up particularly well when mounted in a weak solution of iodine in aqueous potassium iodide.

The mean average length of the dry grains was 37.5 microns, the range extending from 18 microns in *Sporobolus airoides* to 58 microns in *Secale cereale*. A variation of 7 to 10 microns was found in the various samples of a given species. Size alone proved of very little aid in identifying pollens.

In the sunflower family Compositae two distinct types of pollen grains were found, a spherical or oval form with definite spicules and an elliptical form with furrows. The latter form was characteristic of all species of the genus *Artemisia*, the former of all the genera of the tribe Ambrosiaceae. In both forms the surface of the pollen grains appeared slightly granular or roughened as in an orange peeling. Water mounts of all species of *Artemisia* were spherical in general outline yet distinctly three lobed, the lobes representing the exine surfaces between the longitudinal furrows.

The number and size of the spicules were found to be quite variable among all the species of the tribe Ambrosiaceae. Species of some genera, however, resembled each other so closely in spicule characters that it was impossible to distinguish the species in a mixed mount. This was found to be the case also among the genera of Ambrosiaceae. Since in many localities of California the ragweeds (ambrosias), franserias and cockleburrs shed their pollen concurrently, it would be highly doubtful whether one could rely upon specific or even generic identification of the spiculated pollen grains found upon exposed plates. It would be fairly safe to state, however, that all wind blown pollens with spicules belonged to the Ambrosiaceae, as no other strictly wind blown pollens possess this characteristic. All the varieties of *baccharis*, *cosmos*, *coreopsis*, *chrysanthemum*, *gaillardia* and *helianthus* have spicules, but these genera of Compositae being primarily insect-pollinated can scarcely be expected to appear upon a pollen plate.

The third largest family considered was the goosefoot or chenopod (Chenopodiaceae) family. All the species possessed spherical pollen grains. The exine showed distinct round concavities giving the pollen grains the appearance of 'round mesh' golf balls. These concavities or depressions varied in size and number among the different genera and among the species of a given genus. Since a given sample of pollen from a single species of a chenopod showed considerable variation in number and size of these concavities, it was

TABLE I
SUMMARY OF POLLENS STUDIED

FAMILY	NUMBER OF GENERA	NUMBER OF SPECIES	SHAPE WHEN DRY	EXTERNAL MARKINGS AND OTHER FEATURES OBSERVED DRY AND MOIST	
				SHAPE WHEN MOIST	
Acetaceae †Maple	1	2	Elliptical, with furrows	Smooth	Spherical, somewhat 3 lobed
Amaranthaceae †Amaranth	1	4	Spherical, some times irregular	With regularly arranged con- cavities, like round mesh golf balls Slightly granular	Spherical
Betulaceae †Birch	3	4	Polyhedral	Pores at the thickened corners Irregularly spherical	3 to 5 germinal pores
Chenopodiaceae †Chenopod	7	19	Spherical	With concavities or pores, like round mesh golf balls Gran- ular or smooth Starch pres- ent	Spherical
Compositae *Sunflower	11	26	Artemisia — Ellip- tical with fur- rows Other Genera— Oval or spheri- cal	Artemisia—Smooth or very granular Spherical, 3 lobed	Other Genera—Spiculate Spherical or oval
Cruciferae *Mustard	1	2	Elliptical, with furrows	Granules forming regular retic- ulations	Oval
Cyperaceae †Sedge	2	2	Irregularly spheri- cal, oval, pyra- midal Shrunken surfaces	Carex—Smooth, oval Scirpus—Smooth, pyramidal	
Euphorbiaceae *Spurge	1	1	Irregularly oval or spherical Shrunken sur- faces	Smooth and spherical	
Fagaceae †Beech or Oak	5	8	Elliptical, with furrows	Smooth or slightly granular Spherical or broadly ellipti- cal	3 germinal pores
Guttiferae †Silk tassel	1	2	Irregularly spheri- cal	Granular or reticulated Spher- ical	3 germinal pores
Gramineae †Grass	33	68	Irregularly oval, spherical, pyra- midal "Gram- corn like" with shrunken sur- faces	Smooth One germinal pore Starch present Spherical or broadly elliptical or pyram- idal	
Juglandaceae †Walnut	1	3	Irregularly spheri- cal	10 to 14 large pores Scal- loped around edge Spher- ical	
Leguminosae *Pea	1	8	Spherical to oval	With concentric polygons over surface Spherical	
Moraceae *Mulberry	2	2	Irregularly oval to elliptical with shrunken sur- faces	Humulus—Smooth, with 3 pores Spherical Morus—Smooth, with a pore at each pole Spherical	
Myricaceae †Sweet gale	1	1	Polyhedral Shrunken sur- faces	Smooth 3 germinal pores Spherical—somewhat 3 lobed	
Myrtaceae *Myrtle or Eucalyptus	1	2	Equilaterally tri- angular with retusely trun- cated corners	Similar to dry, but swollen	

*Insect and wind pollinated
†Wind pollinated

TABLE I—CONTINUED
SUMMARY OF POLLENS STUDIED

FAMILY	NUMBER OF GENERA	NUMBER OF SPECIES	SHAPE WHEN DRY	EXTERNAL MARKINGS AND OTHER FEATURES		
				OBSERVED DRY AND MOIST	SHAPE WHEN MOIST	
Oleaceae †Olive	1	1	Broadly elliptical	Very granular or reticulated	Spherical	
Palmaeae †Palm	1	2	Elliptical with 1 furrow and acute ends	Smooth	Irregularly spherical	
Papaveraceae Poppy	1	1	Spherical	Smooth color rows	Spherical With 6 narrow furrows	Orange
Pinaceae †Pine	1	-	Spherical with wings	Smooth dry	Shape same as when dry	
Plantaginaceae †Plantain	1	-	Irregularly spherical with shrunken surfaces	Smooth	With 10 to 14 germinal pores	Starch present
Platanaceae †Plane Tree	1	-	Truncated elliptical	Finely granular like an orange peeling	Spherical	
Polygonaceae †Buckwheat	2	6	Irregularly oval to elliptical—variable with irregular folds or furrows	Smooth	Large, abundant starch granules	Spherical
Rosaceae *Rose	1	1	Irregularly spherical to elliptical	Smooth	Spherical	3 germinal pores
Salicaceae Willow	2	3	Salix—Broadly elliptical with 3 furrows Populus—Irregularly spherical	Salix—Reticulated except in furrows	Spherical	
Sparaginaceae †Bur reed	1	1	Irregularly spherical to elliptical with folds or creases	Granular all over surface	Little starch	Little starch
Typhaceae †Cat tail	1	-	Typha Angustifolia—Irregularly spherical or oval Typha Latifolia—In tetrads (groups of four)	T. Angustifolia—Granular all over like an orange peeling	Spherical	
Umbelliferae Parsnip	1	1	Peanut shaped	Smooth	Elliptical with 4 pores at middle, often protruding	
Urticaceae †Nettle	1	1	Irregularly oval or pyramidal, with shrunken surfaces	Smooth	With 3 germinal pores	Spherical

concluded that these characteristics were not suitable criteria for distinguishing the different genera and species of the Chenopodiaceae. The entire surface was either smooth or slightly roughened as in an orange peeling.

Very closely related taxonomically to the chenopods are the Amaranths (Amaranthaceae). Their pollens were similar to those of the chenopods in all observed morphologic features. Since several species of the Chenopodiaceae and Amaranthaceae shed their pollen during the same season and since they are quite likely to be growing in the same vicinity, we might, therefore, ex-

pect to find these pollens mixed on the same pollen plate. But with our present knowledge of pollen morphology it seems quite impossible to identify these pollens from a given mixture of species. In the case of these two plant families the cleavage line of pollen differentiation is broader than a single family, while in the Compositae the cleavage line was within the family and in the Gramineae it was coextensive with the family.

The mean average diameter of the pollen from the species of Chenopodiaceae was 23 microns and for those of Amaranthaceae 24 microns. The range of variation was from 17.87 microns to 30 microns. But since single pollen grains of a given species vary as much as 10 microns in size, it would be presumptuous to say that one could separate the species of these two families by the size of their pollen grains.

All the other pollens studied grouped themselves into 29 families including 33 genera and 59 species, but no single family included a sufficient

EXPLANATION OF FIGURES

Figs 1 to 18 inclusive are photomicrographs of dry pollen grains $\times 720$ showing all the different shapes and gross features found in our studies.

Figs 19 to 34 inclusive are drawings of dry and moist pollen grains showing shapes and more minute features.

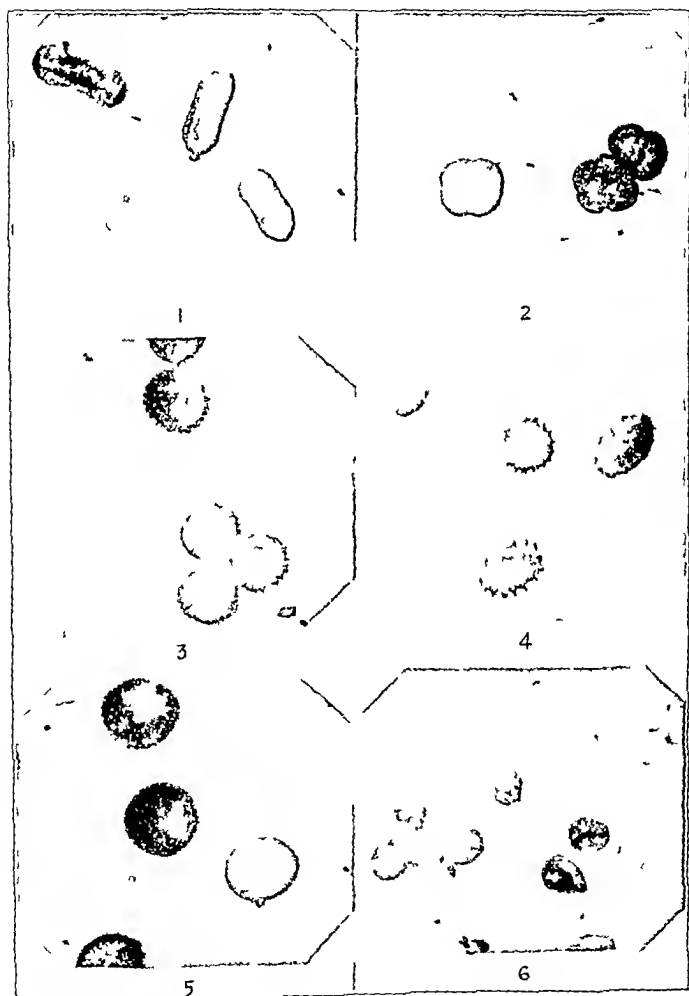
Fig 1—*Foeniculum vulgare* Gaertn., showing peanut-shaped grain. Fig 2—*Typha latifolia* L. pollen grains in tetrads. Fig 3—*Franseria acanthicarpa* Hook., spherical with spicules. Fig 4—*Baccharis pilularis* D. C. oval with coarse spicules almost spinose. Fig 5—*Xanthium canadense* Mill. spherical with very fine spicules almost only coarsely granular. Fig 6—*Urtica gracilis* var. *holosericea* Jepson showing irregularly oval spherical and pyramidal shapes with shrunken surfaces.

Fig 7—*Alnus rhombifolia* Nutt. polyhedral. Fig 8—*Scirpus acutus* Muhl. elongated pyramidal with acute apex. Fig 9—*Phleum pratense* L. with shape of grain of corn with shrunken surfaces and obtuse or rounded corners. Fig 10—*Phalaris canariensis* L., showing single germinal pore. Fig 11—*Phoenix canariensis* Hort. elliptical with a single furrow and acute ends. Fig 12—*Aitenuia campestris pycnocephala* Hall. elliptical with two furrows (one visible) and rounded ends.

Fig 13—*Platanoides occidentalis* L., elliptical with truncate ends slightly granular. Fig 14—*Olea europea* L., broadly elliptical with granular or reticulate surface. Fig 15—*Atriplex rosea* L. like round mesh golf balls characteristic of all Chenopodiaceae. Fig 16—*Amaranthus retroflexus* L., showing similarity to Chenopodiaceae. Fig 17—*Juglans californica* Wats., spherical with scalloped edge in median optical view and numerous pores in surface view. Surface view of pollen grain (upper left hand corner) showing depressions. Fig 18—*Sparganum eurycarpum* var. *greenei* Graebner, spherical (moist) with very granular surface.

Fig 19—*Eschscholtzia californica* Cham. $\times 750$ moist mounts. a, Median optical polar view showing thin perline and six narrow expanded furrow bands. b, Polar surface view showing six narrow furrow bands not reaching the pole. Fig 20—*Salix lasiolepis* Benth. $\times 1400$. Dry mount showing elliptical pollen grain with rounded ends two longitudinal folds and reticulated surface. Fig 21—*Morus alba* L. $\times 1100$. Moist mount median optical view showing thin perline and two polar germinal pores. Fig 22—*Quercus agrifolia* Nee. $\times 1200$. Moist mounts. a, Median optical polar view showing intine protruding through pores in exine. b, Polar surface view showing fine granules and three protruding portions of intine. Fig 23—*Plantago lanceolata* L. $\times 1000$. Moist mount showing distinctly placed germinal pores (10 to 14 to each grain). Fig 24—*Urtica gracilis* var. *holosericea* Jepson $\times 1000$. Dry mount showing irregular shapes and shrunken surfaces and furrows. Fig 25—*Artemisia vulgaris heterophylla* Hall. $\times 1400$. a, Dry mount surface view showing elliptical shape with furrow and smooth surface. b, Dry mount polar surface view showing three narrow longitudinal furrows not reaching the pole. c, Moist mount polar median optical view showing 3 lobed showing 3 crescent-shaped sections of perline united end to end and the intine protruding through the 3 pores found in thin places between two adjacent perline sections.

Fig 26—*Rumex acetosella* L. $\times 1000$. Moist mount showing spherical shape thin perline and abundant large starch grains. Fig 27—*Corylus rostrata* var. *californica* A. DC. $\times 1000$. Moist mount polar median optical view showing three germinal pores through the thickened corners and vacuoles beneath the pores. Fig 28—*Humulus lupulus* L. $\times 1200$. Moist mount surface view showing 3 germinal pores resembling bordered pits of the tracheids of pine. Fig 29—*Alnus rubra* Bong. $\times 1000$. Moist mount polar median optical view showing 5 germinal pores and vacuoles beneath the pores. Fig 30—*Festuca californica* Vasey $\times 1000$. Moist mount showing spherical shape and the single germinal pore. Fig 31—*Pinus radiata* Don. $\times 800$. Moist mount showing lateral wings. Fig 32—*Garrya elliptica* Dougl. $\times 1170$. Moist mounts. a, Surface view showing reticulations. b, Polar median optical view showing 3 germinal pores and perline sections of approximately equal thickness. Fig 33—*Acacia melanoxylon* R. Br. $\times 600$. Dry mount showing concentric polygons over surface. Fig 34—*Eucalyptus globulus* Labill. $\times 1200$. Equilaterally triangular with retusely truncated corner.



pect to find these pollens mixed on the same pollen plate. But with our present knowledge of pollen morphology it seems quite impossible to identify these pollens from a given mixture of species. In the case of these two plant families the cleavage line of pollen differentiation is broader than a single family, while in the Compositae the cleavage line was within the family and in the Gramineae it was coextensive with the family.

The mean average diameter of the pollen from the species of Chenopodiaceae was 23 microns and for those of Amaranthaceae 24 microns. The range of variation was from 17.87 microns to 30 microns. But since single pollen grains of a given species vary as much as 10 microns in size, it would be presumptuous to say that one could separate the species of these two families by the size of their pollen grains.

All the other pollens studied grouped themselves into 29 families including 33 genera and 59 species, but no single family included a sufficient

EXPLANATION OF FIGURES

Figs 1 to 18 inclusive are photomicrographs of dry pollen grains $\times 720$ showing all the different shapes and gross features found in our studies.

Figs 19 to 34 inclusive are drawings of dry and moist pollen grains showing shapes and more minute features.

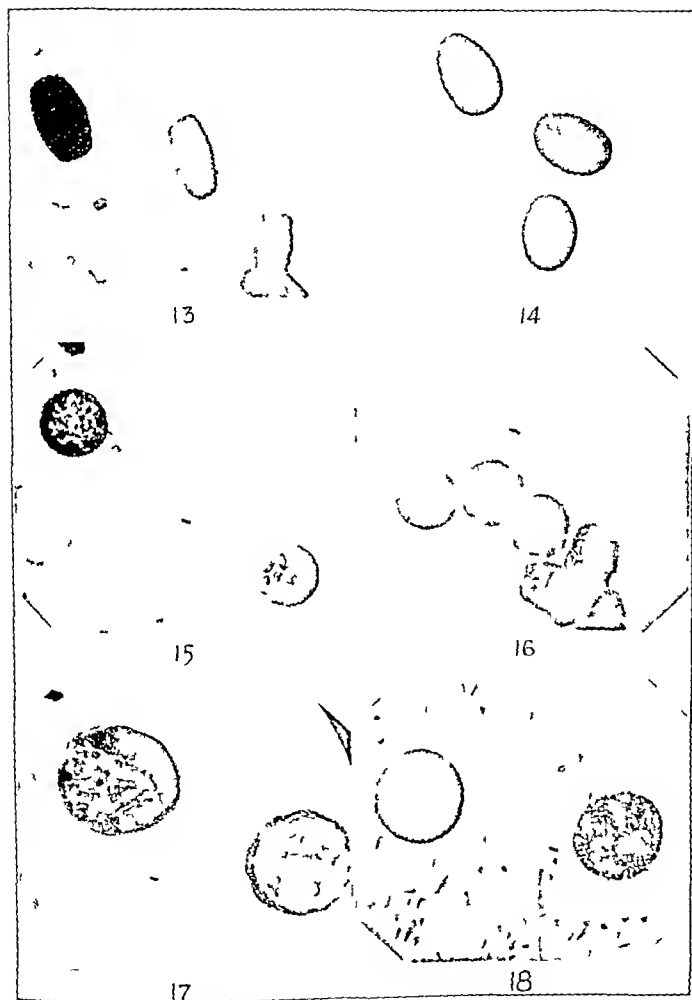
Fig 1—*Foeniculum vulgare* Gaertn showing peanut-shaped grain. Fig 2—*Typha latifolia* L pollen grains in tetrads. Fig 3—*Franseria acanthicaarpa* Hook, spherical with spicules. Fig 4—*Baccharis pilularis* D C oval with coarse spicules almost spinose. Fig 5—*Xanthium canadense* Mill spherical with very fine spicules almost only coarsely granular. Fig 6—*Urtica gracilis* var *holosericea* Jepson, showing irregularly oval spherical and pyramidal shapes with shrunken surfaces.

Fig 7—*Ahus rhombifolia* Nutt polyhedral. Fig 8—*Scirpus acutus* Muhl elongated pyramidal with acute apex. Fig 9—*Phleum pratense* L with shape of grain of corn with shrunken surfaces and obtuse or rounded corners. Fig 10—*Phalaris canariensis* L, showing acute ends. Fig 11—*Phoenix canariensis* Hort elliptical with a single furrow and (one visible) and rounded ends. Fig 12—*Artemisia campestris pycnocephala* Hall elliptical with two furrows.

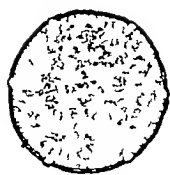
Fig 13—*Platanus occidentalis* L, elliptical with truncate ends slightly granular. Fig 14—*Olea europea* L, broadly elliptical with granular or reticulate surface. Fig 15—*Atriplex rosea* L like round mesh golf balls characteristic of all Chenopodiaceae. Fig 16—*Amaranthus retrofractus* L, showing similarity to Chenopodiaceae. Fig 17—*Juglans californica* Wats spherical with scalloped edge in median optical view and numerous pores in surface view. Surface view of pollen grain (upper left hand corner) showing depressions. Fig 18—*Sparganium eurycarpum* var *greenei* Graebner, spherical (moist) with very granular surface.

Fig 19—*Eschscholtzia californica* Cham $\times 750$ moist mounts *a*, Median optical polar view showing thin perine and six narrow expanded furrow bands. *b*, Polar surface view showing six narrow furrow bands not reaching the pole. Fig 20—*Salix lasiolepis* Benth $\times 1400$ Dry mount showing elliptical pollen grain with rounded ends two longitudinal fold and reticulated surface. Fig 21—*Morus alba* L $\times 1100$ Moist mount median optical view showing thin perine and two polar germinal pores. Fig 22—*Quercus agrifolia* Nee $\times 1200$ Moist mounts *a*, Median optical polar view showing intine protruding through pores in exine. *b*, Polar surface view showing fine granules and three protruding portions of intine. Fig 23—*Plantago lanceolata* L $\times 1000$ Moist mount showing distantly placed germinal pores (10 to 14 to each grain). Fig 24—*Urtica gracilis* var *holosericea* Jepson $\times 1000$ Dry mount showing irregular shapes and shrunken surfaces and furrows. Fig 25—*Artemisia vulgaris heterophylla* Hall $\times 1400$ *a*, Dry mount surface view showing elliptical shape with furrow and smooth surface. *b*, Dry mount polar surface view showing three narrow longitudinal furrows not reaching the pole. *c*, Moist mount polar median optical view 3 lobed, showing 3 crescent-shaped sections of perine united end to end and the intine protruding through the 3 pores found in thin places between two adjacent perine sections.

Fig 26—*Rumex acetosella* L $\times 1000$ Moist mount showing spherical shape, thin perine and abundant large starch grains. Fig 27—*Corylus rostrata* var *californica* A DC $\times 1000$ Moist mount polar median optical view showing three germinal pores through the thickened corners and vacuoles beneath the pores. Fig 28—*Humulus lupulus* L $\times 1200$ Moist mount, surface view showing 3 germinal pores resembling bordered pits of the trichels of pine. Fig 29—*Ahus rubra* Bong $\times 1000$ Moist mount polar median optical view showing 5 germinal pores and vacuoles beneath the pores. Fig 30—*Festuca californica* Vasey $\times 1000$ Moist mount showing spherical shape and the single germinal pore. Fig 31—*Pinus radiata* Don $\times 800$ Moist mount showing lateral wings. Fig 32—*Garrya elliptica* Dougl $\times 1120$ Moist mounts *a*, Surface view showing reticulations. *b*, Polar median optical view showing 3 germinal pores and perine sections of approximately equal thickness. Fig 33—*Acacia melanoxylon* R Br $\times 600$ Dry mount showing concentric polygons over surface. Fig 34—*Eucalyptus globulus* Labill $\times 1200$ Equilaterally triangular with retusely truncated corner.



Figs 13-18.—Taxodium flor.



19a



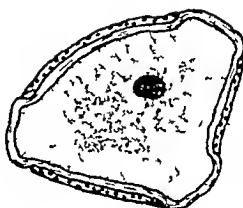
b



20



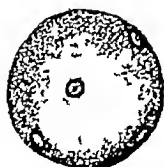
21



22a



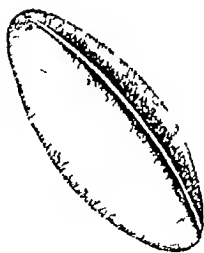
b



23



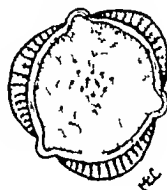
24



25a



b



c



26



27



28



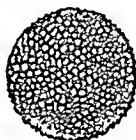
29



30



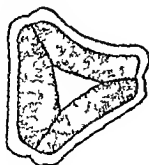
31



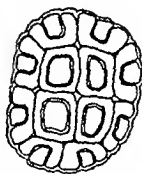
32 a



32 b



33



34

Figs 26-34 — See Explanation of Figures

number of genera or species to warrant more than the specific conclusions given in the summary of families in Table I

KEY TO POLLENS

Shapes and other characteristics refer to dry pollen grains unless stated otherwise

The mean average size of the pollen grains have been obtained from 10 to 100 counts of dry pollen. A variation from 5 to 10 microns may be expected among the grains of the same species

A careful study of the figures and photomicrographs will aid in understanding the terminology used

A knowledge of the plants in the district from which the pollens are secured will aid in verifying the identification made by use of the key

- I Peanut shaped (Fig 1), mean average length 28 microns, summer and autumn
*Foeniculum**
- II In tetrads (Fig 2) (groups of 4), mean average diameter for the tetrads 37 microns.
Typha latifolia
- III With 2 lateral wings (Fig 31)
Pinus
- IV With concentric polygons (Fig 34) all over the surface, spherical to oval, mean average length 40 microns spring
Acacia
- V Equilaterally triangular with retusely truncated corners (Fig 33), not spherical when moist
Eucalyptus
- VI With distinct spicules (Fig 3) or spines, or rarely apparently only coarsely granular (Fig 18)
 - A Oval (Fig 4) or broadly elliptical (Fig 14)
 - 1 Coarsely spinose, mean average length 35 microns
*Helianthus**
 - 2 Coarsely spinose, mean average length 49 microns
*Gaillardia**
 - 3 Distinctly spiculate (Fig 3) but not spinose, oval
 - a Mean average length 30 microns, from cultivated flowers
*Cosmos**
 - b Mean average length 25 microns, from wild shrubs of coast ranges, Oregon southern California, autumn
*Baccharis pilularis**
 - 4 Coarsely granular (Fig 14) or reticulate
 - a Broadly elliptical (Fig 14) or oval, mean average length 27 microns, end view 3 lobed, spring
Olea
 - b Irregularly spherical or oval, end view not 3 lobed
Sparganium
 - B Spherical (Fig 5)
 - 1 From cultivated, insect pollinated flowers, mean average diameter 21 microns, summer and autumn
*Coreopsis**
 - 2 From wild wind pollinated plants, range in mean average diameter 17 to 29 microns, April to November (Ambrosieae tribe of the family Compositae)
 - a Very finely spiculate (Fig 5), apparently only coarsely granular
 - (1) Mean average diameter 29 microns
Xanthum canadense
 - (2) Mean average diameter 24 microns
Franseria bipinnatifida
 - b Distinctly spiculate (Fig 4)
 - (1) Mean average diameter 17 to 25 microns
Ambrosia,
Franseria, Iva and Xanthum spinosum

VII Without spicules or spines

A. Elliptical or oval with regular longitudinal folds (Fig 25) or furrows

1 With one longitudinal furrow (Fig 11) extending from pole to pole ends acute, smooth when moist

a When moist three germinal pores evident, resembling bordered pits (Fig 28) in tracheids of pine, mean average diameter 24 microns

Humulus lupulus

b When moist, germinal pores not evident, mean average diameter 17 microns.

Phoenix

2 With 3 longitudinal furrows (Fig 20) between the poles, only 1 or 2 visible at a given focus

a Surface distinctly reticulated (Fig 20), ends rounded (Fig 12)

(1) Becoming spherical when moist mean average length 27 microns

(a) When moist, reticulated (Fig 14) all over the surface, longitudinal furrow bands not evident

Olea

(b) When moist not reticulated on furrow bands, these very evident

Salix

(2) Becoming oval when moist mean average length 42 microns

*Brassica**

b Surface not reticulated apparently somewhat granular

(1) With truncate ends (Fig 13)

(a) When moist with large abundant starch grains (Fig 26) evident, germinal pores not evident

Rumex

(b) When moist, without large abundant starch grains furrows and pores evident

(aa) Mean average length less than 30 microns, typically 23 microns perine, when moist, apparently of same thickness (Fig 32 b) all around, granular on furrow bands as well as over surface

Platanus

(bb) Mean average length typically 35 to 40 microns, perine when moist thinner in the 3 furrow bands, these expanded smooth not reaching the poles, when moist 3 protruding germinal pores (Fig 22 a) on bands mid polar

Quercus

(2) With rounded ends (Fig 12)

(a) Mean average length 20 microns becoming broadly elliptical when moist with 3 germinal pores mid polar

Castanopsis

(b) Mean average length 24 to 30 microns becoming spherical when moist, often with three prominent lobes

(aa) When moist with large abundant starch grains evident furrows and pores not evident

Rumex

(bb) When moist, large abundant starch grains not evident, furrows evident as bands on perine not reaching the poles (Fig 25 b)

(aaa) Perine when moist in median optical view of unequal thickness appearing as 3 crescents (Fig 25 c) united end to end forming a circle, with a pore at the thin place between 2 adjacent crescent shaped portions of perine

Artemisia

(bbb) Perine when moist in median optical (Fig 32 b) view of nearly equal thickness all around

(11) Pores evident in moist mounts.

Ricinus

(2,2) Pores not evident in moist or dry mounts

Mean average length 30 microns

Acer macrophyllum

Mean average length 35 microns

Acer negundo var californicum

- 2 Without appearance of round mesh golf balls
- a Mean average diameter 14 microns *Adenostoma**
- b Mean average diameter over 20 microns
- (1) Distinctly granular or reticulated (Fig 32 a) all over the surface like an orange, not scalloped around the the edge
- (a) 3 germinal pores evident when moist
- (aa) With irregular folds or creases when dry, mean average diameter 35 microns *Ricinus*
- (bb) Without evident folds or creases when dry
- (aaa) Mean average diameter 29 microns *Garrya elliptica*
- (bbb) Mean average diameter 35 microns *Garrya fremontii*
- (b) Germinal pores not evident when moist or dry
- (aa) With evident irregular folds or creases, mean average diameter 25 microns *Sparganium*
- (bb) Without evident irregular folds or creases, mean average diameter 28 microns *Typha angustifolia*
- (2) Not granular like an orange, but with 10 to 14 large depressions with a pore in each, scalloped (Fig 17) around the edge in median optical view, mean average diameter 36 to 44 microns *Juglans regia*, 36 microns *Juglans californica*, 44 microns
- (3) Smooth rarely apparently granular, without scalloped edges in median optical view
- (a) With 6 narrow longitudinal furrows (Fig 19 b) not reaching to the poles, mean average diameter 29 microns *Eschscholtzia**
- (b) Without narrow longitudinal furrows, mean average diameter 24 microns *Populus*

REFERENCES

- ¹Kerner, Anton, and Oliver, F W The Natural History of Plants, 1893, iii, 95
- ²Koessler, K K and Durham, O C A System for Intensive Pollen Survey, J A M A, 1926, lxxxvi, 1204
- ³Le Maout, Emm, and Decaisne, Jh Traite Generale de Bontanique, 1868, 47
- ⁴Pope, Mary Alice Pollen Morphology as an Index to Plant Relationship, Bot Gaz, 1925, lxxx, 63
- ⁵Scheppepegrell, Wm Hay Fever and Asthma, Philadelphia and New York, 1922, Lea and Febiger
- ⁶Wodehouse, R P Morphology of Pollen Grains in Relation to Plant Classification, Jour N Y Bot Gard, 1926, xlvii, 145

TREATMENT OF ASTHMATIC PATIENTS AFFECTED BY PROTEIN OF THE EPIDERMAL GROUP*

By ZELLA WHITE STEWART M.D., IOWA CITY, IOWA

THIS report contains the results of a careful study of a group of 202 cases. How generally they can be applied is not considered. The conclusions may be summarized as follows:

- 1 That a diagnosis has been made in every case of asthma by testing repeatedly and thoroughly
- 2 That a majority of the cases of asthma in this group are sensitive to feathers
- 3 That many asthmatics are sensitive to very slight contacts
- 4 That specific desensitization has not brought about permanent results in many cases
- 5 That absolute relief can be secured when all offending proteins have been found, and the proper evaluation assigned to each as to the extent it is entering into the case as a causative factor and all such eliminated from the food and surroundings

I have tested in all 202 cases prior to May 1, 1926. All of these were clinically cases of allergy and all have given positive skin reactions. During this same period I have made complete tests in six cases that clinically were not cases of allergy. This was done in order to rule out sensitivity. They were clinically cases of sinus infection, chronic bronchitis, dermatitis, digestive disturbance and frequent colds.

The following case will illustrate some of the difficulties met in many cases in securing positive skin reactions:

Mrs. H. C. aged 42 was first seen in July 1922. She gave a history of hay fever as a child and asthma since 18 years of age. At this time she was never free from marked asthmatic bronchitis, wheezing and shortness of breath on exertion and had frequent severe attacks of asthma. Her condition was complicated by exophthalmic goiter. Due to the malnutrition accompanying the same her skin was dry and unhealthy.

During 1922 she was tested three different times with all available proteins by the scratch tests without results. She spent the winter of 1922-23 in Arizona, hoping her bronchitis would improve in a milder climate so that she could submit to an operation. Her asthma was worse on her journey south and during her stay there she was confined to a hospital. Her chances of contacts with proteins in a hospital were limited to feathers, wool powder and foods.

In the summer of 1924 she was operated on for goiter. The report from the hospital in regard to her asthma was as follows: It seems to us that rather than being a foreign protein affair, it is more of an asthmatic bronchitis as most of her asthmatic attacks

Presented before the American Association for the Study of Allergy, Washington, D. C.
May 17, 1926

Five cases or 4 per cent discontinued treatment and have had no improvement. This in some cases was due to the advice of their physicians who remarked that the work in allergy was still in an experimental stage.

In five cases or 4 per cent I have no knowledge of their present condition.

I keep all my cases under supervision for at least two years, if possible or until they have gone free of trouble for one year. Usually the first year they report weekly, either in person or by letter, whether they are receiving desensitization or not, and the second year monthly. By the time they have gone one year free from symptoms they have acquired the knowledge of what they must avoid.

habiting the same. After his efforts he spent most of the night struggling for his breath. He can travel and stay at hotels if the pillows are removed but he cannot spend a night at the home of his mother in law which is an old house with many feather beds.

In many patients who have not been desensitized but have had two years supervision and have learned what contacts to avoid, the results are as good.

To secure results with pollen the necessity of several years of treatment is well established. Better results might be obtained if the same procedure were carried out in the case of animal emanations.

Better results are obtained with men than with women where the trouble is due to animal emanations found in homes on account of less opportunity of contact to small amounts of protein left in curtains and rugs even after everything has been removed.

Perhaps years of freedom with the accompanying improvement in general health will decrease this sensitiveness. This I think will occur especially in children.

Even if desensitization does not accomplish all that could be wished for, I am thoroughly convinced that every asthmatic, regardless of the duration of the trouble, can be absolutely relieved when all offending proteins have been discovered. It may take months of testing and retesting and the use of both scratch and intradermal method. It will mean close inspection of surroundings and supervision for a long period.

END RESULTS

Of this group of 116 cases where the primary cause has been due to animal emanations, 67 or 58 per cent have been free from all symptoms for at least one year. This has been due to the thorough eliminations of the offending proteins and to the knowledge they have acquired of their individual sensitiveness to contacts. Many of these have been desensitized to one or more proteins. Close supervision and observation of these cases indicates that freedom from contacts has brought about these results. This group has not only been free of symptoms but has shown improvement in general health and freedom from colds.

Twenty two cases or 19 per cent have had about 75 per cent improvement. They do not have asthmatic attacks but still show protein irritation by wheezing, shortness of breath on exertion and by frequent colds and bronchitis. Many of these cases are free from all symptoms for several months. They are still under supervision and many of them are having their tests checked over.

Three cases or 2 per cent have had no improvement. They have been under treatment for two years and have given satisfactory reactions but their condition is proof that all factors have not been discovered or all contacts found.

Three cases or 2 per cent have been discharged for the reason that it was impossible to get their cooperation in eliminating the causes of their trouble.

Eleven cases or 9 per cent discontinued treatment or supervision after complete tests were made. They report freedom from attacks but slight symptoms of irritation. They are satisfied with the results.

Five cases or 4 per cent discontinued treatment and have had no improvement. This in some cases was due to the advice of their physicians who remarked that the work in allergy was still in an experimental stage.

In five cases or 4 per cent I have no knowledge of their present condition.

I keep all my cases under supervision for at least two years, if possible or until they have gone free of trouble for one year. Usually the first year they report weekly, either in person or by letter, whether they are receiving desensitization or not, and the second year monthly. By the time they have gone one year free from symptoms they have acquired the knowledge of what they must avoid.

STUDIES IN ASTHMA*

I A CLINICAL SURVEY OF 1074 PATIENTS WITH ASTHMA FOLLOWED FOR TWO YEARS

By FRANCIS M RACKEMANN, MD † BOSTON

AS A preliminary to a more intensive study of asthma, an attempt has been made to follow all those patients seen at the Massachusetts General Hospital and in private practice, whose first visit was prior to January 1, 1925, and in whom, therefore the follow up note would cover a period of at least two years

The object is twofold first to study the clinical classification of the patients, partly to compare this present grouping with previous classifications and partly to see whether the preliminary classification would be confirmed by subsequent observations of the patients. The second object is to study the results of treatment and any light which these results of treatment or clinical management might throw upon the mechanism of the asthma

The total series comprises 1514 patients. Of these 1074 patients (70 per cent) have been either heard from or seen again between January 1, 1927, and May 1, 1927 and the end results thus obtained are presented herewith

This series of 1074 cases has been divided into three general groups extrinsic, intrinsic and unclassified asthma. A brief description of terms and methods of diagnosis is necessary. 'Extrinsic' is the term applied to those cases hypersensitive to some foreign substance outside of the body, and who have asthma on exposure to or contact with it. The term "intrinsic" implies that the essential cause of the trouble is inside of the body. The miscellaneous "unclassified" group includes the cases with unknown cause. Each of these main groups has been divided into subclassifications. Similar classifications of the types of asthma have been made previously by the author¹ by Cooke,² and by others,³ partly on the basis of the causative agent and partly on the basis of the end results of treatment. These classifications are always unsatisfactory in some degree but are necessary for any approach to the study of the mass of data which accumulates

The diagnosis of the cause of asthma and the classification of the particular case must continue to rest chiefly on the history of the disease in the particular patient and to be dependent upon the circumstances under which the attacks of asthma have occurred. Thus if the asthma was entirely relieved when the patient moved from one environment to another, such a move clearly suggests an extrinsic factor probably in some dust which was causing the attacks in the first place and from which the patient escaped by the move

*The expenses of this investigation were met by an anonymous donation known as the M G H Asthma Fund

†From the Medical Services and Anaphylaxis Clinic of the Massachusetts General Hospital

Read before the American Association for the Study of Allergy at Washington D C on May 21 1927

Skin tests by the scratch method and by the intradermal method, and frequently by both at the same time, have been useful in two ways first, to confirm the diagnosis as suggested by the history, and second, to point out other possibilities, which, however, must always await confirmation by further cross-examination of the patient's story, or perhaps by such a clinical experiment as a change in residence, a restriction of diet or an elimination of some supposedly offending substance

In case the results of the skin tests cannot be confirmed in this way, the suspected substances have been disregarded as a cause of the present trouble and considered simply, with Cooke, as "potential" causes of asthma. In other cases with a positive history, but with negative tests, the diagnosis has been based upon this history and not excluded because the skin tests were negative

The results of treatment made available by the follow-up system have provided another means of confirming the diagnosis and classification. Methods of treatment have been selected according to the probable cause of asthma, as determined by the preliminary study. Thus in the extrinsic group, the offending foreign substance has been eliminated, perhaps by removing some one article like the cat, the feather pillow or a substance met with in the occupation or perhaps by a more radical procedure, like a change in climate, residence or work. In case the offending substance could not be eliminated, attempts to "desensitize" the patient to it have been made

TABLE I
1074 CASES OF ASTHMA
GROSS CLASSIFICATION

DECADE OF ONSET	0 9	10 19	20 29	30 39	40 49	50 59	60	TOTAL
Extrinsic Asthma	162	75	96	55	27	9	1	425
Intrinsic Asthma	128	59	78	97	75	43	19	499
Miscellaneous Unclassified Asthma	23	32	28	31	27	7	2	150
Total	313	166	202	183	129	59	22	1074

In the intrinsic group, treatment has consisted of removing foci of infection and in improving the general condition of the patient by changes in dietary, by regulation of rest and exercise and by removal of those factors which might cause irritation to mind and body. In fact, this attention to "general hygiene" has always been a very important feature of the treatment. In the cases of bacterial asthma and in many others where repeated respiratory infections have been important as primary or as secondary causes of asthma, vaccines, both stock and autogenous, have been employed to produce an immunity by specific or nonspecific means

Table I shows the numbers of cases in each main group, arranged according to the decade of onset of the asthma. As originally pointed out by Walker,³ the percentage of extrinsic asthma, which is high among children, bears a constantly diminishing relation in the older decades to the cases of intrinsic asthma and to the total series. Though Table I does not give the figures, males and females occur about equally throughout the series, except that among those with an early onset of asthma, males predominate

Extrinsic asthma is the easiest group to study. Chart I shows a dot for each patient, still under treatment plotted opposite his present age shown by the vertical figures at the left and placed in the proper decade of onset of his asthma according to the horizontal figures at the bottom. The circles represent those patients asthma free for at least two years* and the crosses represent deaths whether from asthma or from any other cause. Chart I shows a definite grouping of present young people whose asthma began in childhood and here the number of cures is of particular interest. It is

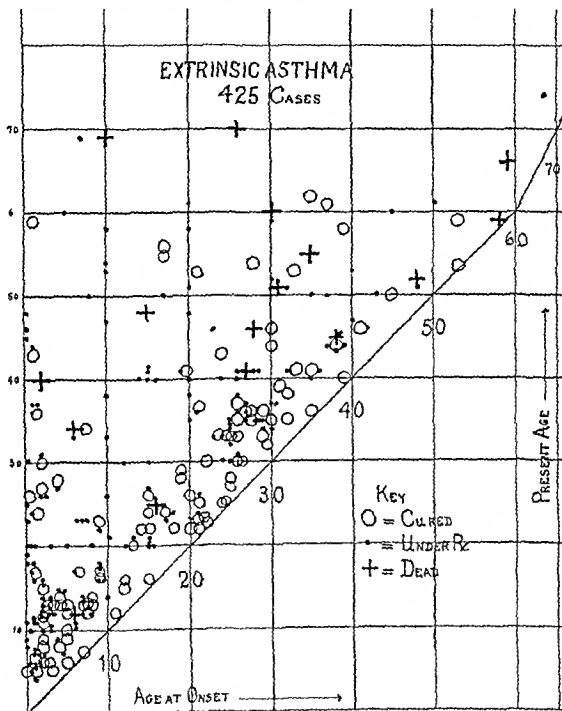


Chart I

worthy of note that asthma of the extrinsic type rarely begins after the age of 40 (Compare Chart II later)

The subclassification of these extrinsic cases is shown by Table II. Here we have groups of cases with the end results as obtained by the follow up questionnaire. The sex incidence, the percentage of positive family histories and the total number of positive skin reactions are also included.

Later in this paper the word *cured* in quotation marks is used to denote patients asthma free for two years or more without treatment. The author fully appreciates the danger of using such a word and agrees with the comments recently made by M. H. Kahn.

Table II shows asthma due to pollens, due to animal dusts and due to unidentified causes. The patients with "pollen asthma" include only those who come without particular reference to their hay fever. Obviously, however, true pollen asthma can only be an aggravation of hay fever, and if it is true that one-third of all patients with hay fever wheeze during the pollen season and therefore have asthma, as Rackemann⁵ pointed out, the present figure is far too low.

TABLE II
EXTRINSIC ASTHMA
CLASSIFICATION AND RESULTS

	"CURED"	IMPROVED	SAME	DEAD	TOTAL	PER CENT		
						FEMALE	POSITIVE FAMILY HISTORY	POSITIVE SKIN TESTS
Pollen asthma	13	44	8	5	70	31	45%	68
Pollen asthma infected	5	23	10	1	39	23	40%	35
Summer asthma, negative tests	10	10	10	2	32	15	10%	0
Animal asthma	22	32	5	1	60	32	60%	60
Animal asthma, infected	0	11	2	0	13	6	61%	13
Extrinsic mixed and unidentified	22	80	13	6	121	64	45%	121
Mixed and unidentified with negative tests	18	14	5	0	37	16	48%	0
Extrinsic specials*	17	26	9	1	53	20	54%	52
Total	107	240	62	16	425	207	47%	349
Per cent of total	25%	56%	15%	4%	100%	49%	47%	82%

*These include

Dust—Feathers 10 orris 3 cotton dust 1 hops and malt 1 fish glue 1 dyes 1 Formu
lin 1 wheat (as dust) 5
Foods—Eggs 12 wheat 4 fish 3 milk 2 mixtures 9

The poor results of treatment in eight cases of the simple type and in ten cases of the infected type represent the difficulties of treatment and indicate also that the presence of infection is a severe obstacle to treatment. The fact that the total number of positive skin tests in the two groups falls short of the total number of patients by six is because skin tests were not done. But these six cases have been included here, rather than in the next group, simply because the story of asthma occurring only during the rag weed season was so definite.

A group of 32 cases is designated "summer asthma with negative tests." Here the cause of trouble is unidentified, except in so far as the history shows the occurrence of asthma only in well-defined seasons, which for each case remain quite the same from year to year. In spite of skin tests, by the scratch method and the intradermal method, as well as by direct application of what appeared to be the offending pollen, to the conjunctival sac, it has been, so far, quite impossible to prove that pollens are the cause. Moreover, the results of treating these patients, either with strong pollen extracts or with vaccines have been quite unsatisfactory. Meantime, nevertheless, ten patients are "cured." Six of these were children who became asthma free without particular treatment other than directions for improving the general hygiene, one of the adults had vaccines, another has moved to California and the two others have become symptom-free without knowing why.

The low percentage of positive family history in this group of summer asthma is interesting, particularly as the contrast with other extrinsic groups is so great.

The group of animal asthma shows a better percentage of good results than any other. Among the unimproved cases there are a great many patients who claim to have been cured of their asthma but who have not been relieved of symptoms long enough to be definitely counted in the cured group. It is of interest to note in connection with the cases of simple animal asthma, that many patients are free from asthma so long as they keep away from the offending animal but as it is entirely beyond their control to keep animals from all the places which they frequent they still have symptoms whenever they cannot avoid the particular contact. In the majority of cases, contact can be avoided for most of the time and the slight change in environment is easier and more certain of results than any direct treatment. The few cases still unimproved are those who have been unable to make the necessary changes to escape from the offending substance and whose specific treatment has been unsuccessful.

In the group of patients whose asthma is due to unidentified causes the number is large. The diagnosis has been applied chiefly to those whose story shows a clear relationship between their asthma and their environment. Many of them have other evidences of hypersensitiveness like eczema or hay fever; most of them give skin reactions to house dusts and frequently to other substances at the same time. The term mixed and unidentified must suffice for the present.

Table II includes a second group of cases whose skin tests were quite negative but whose stories were so definite as to preclude a cause of asthma other than some obscure extrinsic factor. For example:

A woman of about 40 had had asthma since the age of 20. Her father had had hay fever. Her attacks had occurred at all seasons of the year and she had lived all her life in the same house in Belmont. Since her skin tests were repeatedly negative and since the story was of trouble which was so persistent she was at first classified as bacterial asthma in the intrinsic group. But treatment was of little benefit. Recently she wrote that for two years she had remained practically free of all trouble from her asthma and luckily she added this note: "Please note that two years ago I moved from Belmont to Brookline." This note of course has changed her classification and she is now placed in the extrinsic group among those cases of mixed and unidentified asthma with negative tests.

The 37 cases listed as mixed and unidentified with negative tests are all of this type. The fact that in five cases the asthma is still unimproved is explained in one instance by the patient going to Italy and having no further asthma but on return having trouble again; in the second instance, by a marked improvement on moving to California but with a recent return of asthma out there; in the third instance by the fact that the asthma which at first was associated only with a farm barn is now not limited to such exposure and is much more chronic; in the fourth instance by the onset being shortly after moving to a farm persisting while there but with comparative freedom from symptoms when in cities; and in the fifth instance, by the fact that although the patient was temporarily improved by injections

of feather extract (to which he gave a slight skin reaction) he is entirely free on various occasions, one of them being when he was in the hospital ward. In this unidentified group, with negative tests, it should be noted that the incidence of a positive family history is close to the average percentage.

The group designated "extrinsic specials" includes a number of patients in which the cause was well defined and different from any of the other groups. The rather small number of cases, in which the asthma was due to such things as feathers and oint powder, is surprising in view of so many enthusiastic reports in the literature which give the impression that these substances are quite common as causes of asthma. The many young girls who develop a sensitiveness to oint powder manifest this by vasomotor rhinitis and not by asthma in most cases.

Of the total, 53 cases, under this heading, it is interesting that foods produce asthma in 30 cases. All of these are in small children except for 8 adults, each of whom is of special interest. Two were college students, so sensitive to eggs as to find it necessary to avoid all cake and puddings in their dietary. Another was a young surgeon, so sensitive to fish and fish glue that he could not lick a postage stamp without swelling of his tongue and mouth. He also had asthma from the dust of dried fish glue. The fourth, a storekeeper, was sensitive to fish and eggs. Two women were sensitive to fish, and one of them was also sensitive to a great variety of other foods, the most common of which were orange, apple and celery. The other two patients, a man and woman, were sensitive to wheat as a food. All of these recognized their sensitiveness to certain foods before the test and all were free of symptoms so long as they avoided the offending articles.

TABLE III
INTRINSIC ASTHMA
CLASSIFICATION AND RESULTS

	"CURED"	IMPROVED	SAMP	DEAD	TOTAL	FEMALE	PER CENT POSITIVE FAMILY HISTORY	POSITIVE SKIN TESTS
Bacterial asthma	25	128	42	7	202	98	36%	44
Bacterial asthma in children	34	49	5	2	90	24	48%	18
Reflex asthma, not N & T	18	35	11	2	66	41	33%	10
Reflex asthma, N & T only	10	20	7	3	40	22	43%	2
Cardiac asthma	1	13	10	21	45	20	40%	7
Bronchitis and emphysema	3	14	29	10	56	18	21%	3
Total	91	259	104	45	499	223	37%	84
Per cent of total	18%	52%	21%	9%	100%	45%	37%	17%

One case with negative tests was included in this group. He was a child of two who developed asthma and urticaria on every attempt to take cow's milk or goat's milk. At one time another doctor did find a slight reaction to the albumen in cow's milk. This child is now six years old and is still

unable to take milk in any form. Unfortunately no recent tests have been made.

Intrinsic asthma is the term applied to the cases where the cause is inside the body (Chart II for intrinsic asthma corresponds in every way to Chart I for extrinsic asthma). The author feels that such a diagnosis is correct in several fairly definite groups of cases and that the use of the term need not in any sense be forced simply because of the inability to identify an extrinsic

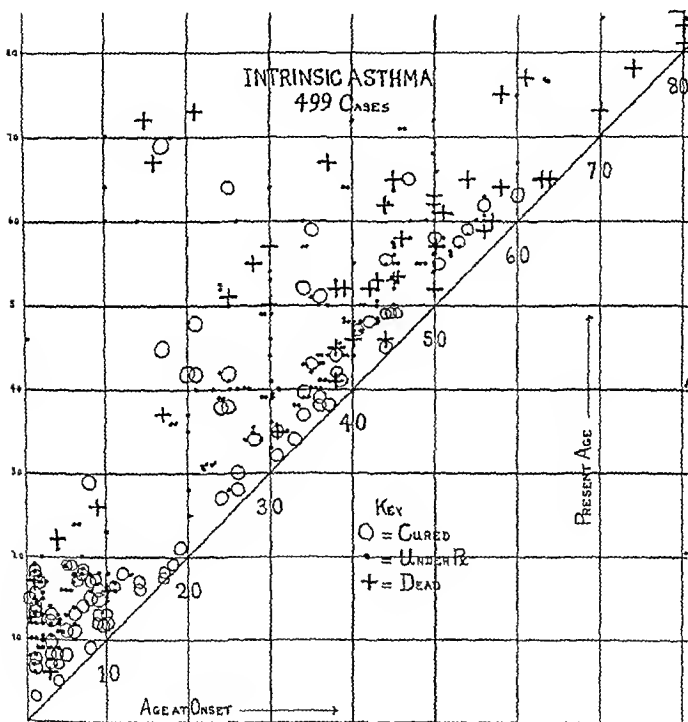


Chart II

cause. The intrinsic group includes various subclassifications as shown by Table III.

"Bacterial asthma" applies to those cases whose asthma is apparently dependent upon an infection in the upper or lower respiratory tract. 'Reflex asthma' indicates that the cause of trouble is either outside of the respiratory tract entirely, as in bad teeth, cholecystitis or constipation, or

that, if inside of the respiratory tract, the lesion is well defined, as in an infected sinus or in infected tonsils

The group of bacterial asthma is of particular interest. Here is a considerable group of patients whose asthma occurs only after some acute respiratory infection including common colds, and this occurrence may happen at very long intervals, perhaps only twice a year, the important point being that the patient is living in the same environment with the same occupation and on the same general diet at all times, facts which would seem to exclude an exposure to any new foreign substance as a cause of the attack. In other cases the evidence of the initial cold is less definite, but again, the intervals between the attacks are long, each one runs a limited course and dependence upon some respiratory infection seems obvious. Individuals with this type of asthma are perfectly well between attacks and show no abnormalities in the chest either on physical examination or by x-ray. Study of the conditions in the nose and throat is in progress.

"Winter asthma" has been used in the past as a convenient term to define an artificial group of these bacterial cases whose trouble begins with the first cold of autumn and persists, with ups and downs, till the warm weather of spring.

The separation from the main group of "bacterial asthma in children" is obviously artificial and is made largely to demonstrate the considerable number of children who have been asthma-free for two years and another considerable number who are improved. Attention should be called to the figure of only twenty-four females in this group of ninety children. In both groups of bacterial asthma are positive skin tests, and yet the diagnosis has been made in spite of this finding. Many of these patients react to house dust and a few of them to such other substances as feathers, dog hair, goat hair, oint powder, etc., but these tests were, with a few exceptions, never well marked, and all except 22 were obtained by the intradermal method, which, in our hands, gives confusing readings in many cases. The real reason to exclude these tests, however, depends upon the fact that in none of these patients was it possible to show that the attacks of asthma were dependent upon exposure to the particular substance. On the other hand, it was perfectly reasonable and logical to explain the attacks on the basis of a respiratory infection. These positive skin tests may well represent a general characteristic of the asthmatic patient rather than a hypersensitiveness which is clinically important.

The designation "reflex asthma" is applied to those cases who have a focus of infection, which may be in the nose and throat or elsewhere, such as in the teeth or in the gastrointestinal tract, and which, because of improvement following treatment can be regarded as a direct cause of asthma. Included in this group are patients with obesity and other general disturbances, which seem to have a very definite bearing on the cause of the trouble, moreover, the study of end-results has clearly justified such a classification. For example six cases of asthma and obesity seemed to be better of their asthma when their weight was reduced, but whether this can be interpreted as cause and effect is perhaps doubtful.

Analysis of 66 cases in which the cause of asthma appears to be a 'reflex' from disturbances outside of the nose and throat, shows, in addition to the six cases of obesity, thirteen cases in which teeth were important, in eight cases the gastrointestinal tract, in four cases nerves," in three cases syphilis, in one case pregnancy and finally in thirty one cases the cause of asthma is designated simply as poor hygiene. Poor hygiene includes such items as a poor or unwise dietary schedule a lack of proper fresh air and exercise, an insufficient intake of fluids, overexertion and continued nervous strain without proper rest periods (or even simply continued loss of sleep, not accompanied by exertion and nervous strain), constipation or indigestion caused by indiscretions in diet all of which have been relieved by following the simplest directions for general hygiene which have been the only treatment and have produced rather amazing results in the majority of cases.

In this group of reflex asthma eleven patients are unimproved, but in spite of that they are here included because the treatment recommended to relieve the particular trouble which seemed so clearly the cause of the asthma, was not carried out. Thus four obese patients lost much of their asthma when they lost weight but when they regained weight, asthma returned.

Of the forty patients with reflex asthma due to nose and throat pathology," thirty eight had a focus of infection in the sinuses nine in the tonsils, and six had a vasomotor rhinitis. Forty six previous operations were done on these patients (only two of which were tonsillectomies). After examination here further operations were advised on thirty two patients, and eight of these had more than one operation (five being tonsillectomies). The other eight patients had less radical local treatment such as cauterization or irrigation, but without surgical intervention. That the designation 'reflex' is justified is shown by the results since of the forty patients ten have become asthma free and twenty are improved. The seven patients included in the group but who are now unimproved did experience temporary relief after operation, but all of them have chronic and extensive sinusitis at the present time.

The diagnosis of cardiac asthma has been made in those patients whose asthma bore an unusual relation to exertion and who gave a rather characteristic story of good nights without being awakened by the nocturnal paroxysm so typical of other types of asthma. In addition the group includes those who present some definite abnormality in the size and action of the heart as well as those whose blood pressure is constantly elevated. In this series, however we have not seen the sudden seizure of dyspnea associated with struggling and excitement which Pratt¹⁴ has so graphically described as characteristic of cardiac asthma. The many patients who have died and the great number whose asthma is unimproved confirm the impression of cardiac damage.

"Chronic bronchitis and emphysema" is the designation of another group which is closely related to the group of cardiac asthma. The distinguishing feature lies in the evidences of faulty oxygen absorption as shown by chronic cyanosis of the lips and fingers and of severe and persistent asthma as shown by a barrel shaped chest in many cases and by markedly high pitched breathing with diminished intensity in all of the cases. The presence of chronic

bronchitis, with cough as an important symptom and with sputum which is considerable in amount and thick yellow in quality, also distinguishes the group. Here too the number of deaths and poor results are both large.

Miscellaneous unclassified cases are included in Table IV. It is gratifying to find that there are only 150 cases in the group. Included under the heading, however, are three special groups worthy of comment. The term "chronic severe asthma" is purely artificial, but is applied to a characteristic type of patient seen not infrequently in the wards of the hospital. Such a patient is most often a man, with an onset of his asthma in the thirties or before, who has had asthma usually for three to five years, rarely for ten or fifteen years, and frequently dates the onset from the time of a severe nervous strain, com-

TABLE IV
MISCELLANEOUS UNCLASSIFIED ASTHMA AND SPECIAL GROUPS

	"CURED"	IMPROVED	SAME	DEAD	TOTAL	PER CENT		
						FEMALE	POSITIVE FAMILY HISTORY	POSITIVE SKIN TESTS
Miscellaneous unclassified	15	40	34	16	105	56	35%	34
<i>Special Groups</i>								
Chronic severe asthma	0	2	15	5	22	6	36%	5
Fatal asthma	0	0	0	10	10	10	50%	5
Asthma and tuberculosis	0	3	1	9	13	4	56%	5
Total	15	45	50	40	150	76	38%	49
Per cent of total	10%	30%	33%	27%	100%	51%	38%	39%

monly in the war. The asthma in these men is of maximum severity, requiring doses of adrenalin every two or three hours, it is not relieved by a stay in the ward, nor is it much changed on returning home. These patients are always thin and pale, they sweat easily, cannot eat, are restless and very uncomfortable. Physical examination frequently shows sinusitis of extensive type. The lungs are emphysematous and the heart action is rapid, with low blood pressure, but the abdominal organs and urine are normal, and the blood calcium, blood nitrogen and blood sugar are within normal limits. Skin tests are almost always negative. Treatment of these poor unfortunates is most unsatisfactory. While the temptation to regard them as having "nervous asthma" is great, yet attempts to relieve them by psychic treatment have been unavailing.

The second special group is "fatal asthma," which has previously been described by Rackemann⁷ as occurring among middle aged women who tend to slight obesity and whose asthma has occurred largely in definite attacks, but eventually has led to more severe attacks, the last of which was fatal. So far ten cases have been collected.

"Asthma and tuberculosis" have been associated in thirteen patients, most of whom had tuberculosis in an advanced stage, and have died of it. The association of the two diseases is interesting because the incidence of asthma among large groups of tuberculous patients is considered to be small. The patients here had asthma which was often severe, but only in three of the cases was it possible to demonstrate any relation between the severity of the

asthma and the activity of the tuberculous infection. No claim for an etiologic relationship is made. Five of the patients tested had positive skin tests, and only one of these was improved by the treatment indicated by the tests.

Table V shows the gross results of treatment in the entire series. Twenty per cent of all the cases have remained free of asthma for at least two years since their last treatment. These 213 'cured' cases will be the subject of a special study to follow. On the other hand, another 20 per cent have remained quite unrelieved of their trouble and 10 per cent have died from various causes. The incidence of death seems to be a rather high figure. In only twenty one of the cases was the death directly due to asthma, for in thirteen it was due to acute respiratory infections, in twenty one to cardiac conditions, in nine to tuberculosis, and in the remainder to various other conditions, for the most part organic, with two violent deaths, and nineteen concerning whom we have no information further than that the patient is no longer living.

In the entire series of 1074 cases shown in Table V, males are rather more numerous than females, but the difference is not striking. A positive family history was obtained in 42 per cent of all the cases. While at least 45 per cent

TABLE V
1074 CASES OF ASTHMA FROM RESULTS

	CURED	IMPROVED	SAME	DEAD	TOTAL	PER CENT		
						FEMALE	POSITIVE FAMILY HISTORY	POSITIVE SKIN TESTS
Extrinsic asthma	107	240	62	16	425	207	47%	349
Intrinsic asthma	91	259	104	45	499	223	37%	84
Miscellaneous unclassified asthma	15	45	50	40	150	76	38%	49
Total	213	544	216	101	1074	506	42%	482

of the patients gave positive skin tests it should be noted that certain cases in the extrinsic group failed to give positive skin tests, while certain cases in other groups did show positive skin reactions that were neglected as an important factor in the cause of the asthma. A study of these irregular skin tests is contemplated.

DISCUSSION

This paper presents briefly the gross results of a study of 1074 patients with asthma, all of whom were seen for the first time at least two years ago, so that the final notes showing the condition of these same patients at the end of two years or more are available for a general survey of their progress.

The two charts showing the decade of onset, the present age and the present status of 425 patients in the extrinsic group and of 499 patients in the intrinsic group, demonstrate clearly the pleomorphic character of asthma as a disease and give further support to the conception that asthma is a symptom based on a variety of causes rather than a disease with a single etiology.

It was hoped that a survey of such magnitude would demonstrate certain natural tendencies of the symptom asthma to come and go and that it would point to a mechanism which might explain the natural history of the disease.

bronchitis, with cough as an important symptom and with sputum which is considerable in amount and thick yellow in quality, also distinguishes the group. Here too the number of deaths and poor results are both large.

Miscellaneous unclassified cases are included in Table IV. It is gratifying to find that there are only 150 cases in the group. Included under the heading, however, are three special groups worthy of comment. The term "chronic severe asthma" is purely artificial, but is applied to a characteristic type of patient seen not infrequently in the wards of the hospital. Such a patient is most often a man, with an onset of his asthma in the thirties or before, who has had asthma usually for three to five years, rarely for ten or fifteen years, and frequently dates the onset from the time of a severe nervous strain, com-

TABLE IV
MISCELLANEOUS UNCLASSIFIED ASTHMA AND SPECIAL GROUPS

	"CURED"	IMPROVED	SAME	DEAD	TOTAL	PER CENT		
						FEMALE	POSITIVE FAMILY HISTORY	POSITIVE SKIN TESTS
Miscellaneous unclassified	15	40	34	16	105	56	35%	34
<i>Special Groups</i>								
Chronic severe asthma	0	2	15	5	22	6	36%	5
Fatal asthma	0	0	0	10	10	10	50%	5
Asthma and tuberculosis	0	3	1	9	13	4	56%	7
Total	15	45	50	40	150	76	38%	49
Per cent of total	10%	30%	33%	27%	100%	51%	38%	39%

monly in the war. The asthma in these men is of maximum severity, requiring doses of adrenalin every two or three hours, it is not relieved by a stay in the ward, nor is it much changed on returning home. These patients are always thin and pale, they sweat easily, cannot eat, are restless and very uncomfortable. Physical examination frequently shows sinusitis of extensive type. The lungs are emphysematous and the heart action is rapid, with low blood pressure, but the abdominal organs and urine are normal, and the blood calcium, blood nitrogen and blood sugar are within normal limits. Skin tests are almost always negative. Treatment of these poor unfortunates is most unsatisfactory. While the temptation to regard them as having "nervous asthma" is great, yet attempts to relieve them by psychic treatment have been unavailing.

The second special group is "fatal asthma," which has previously been described by Rackemann⁷ as occurring among middle aged women who tend to slight obesity and whose asthma has occurred largely in definite attacks, but eventually has led to more severe attacks, the last of which was fatal. So far ten cases have been collected.

"Asthma and tuberculosis" have been associated in thirteen patients, most of whom had tuberculosis in an advanced stage, and have died of it. The association of the two diseases is interesting because the incidence of asthma among large groups of tuberculous patients is considered to be small. The patients here had asthma which was often severe, but only in three of the cases was it possible to demonstrate any relation between the severity of the

The classification as presented is not perfect because our knowledge of the underlying mechanism is still lacking but the fact that reclassification of patients in the same clinic yields figures for the different groups which are quite comparable from time to time indicates that the method is reasonably accurate and clinically useful.

REFERENCES

- ¹Rackemann, F. M. A Clinical Classification of Asthma. *Am Jour Med Sc* 1921 cxvii 802.
 Cooke, Robert A. Studies in Specific Hypersensitiveness. New Etiologic Factors in Bronchial Asthma. *Jour Immunol* 1922 vii 147.
²Walker, I. C. Bronchial Asthma: a Clinical Study of 400 Patients with Boston Med and Surg Jour 1918 cxviii 288.
 Larson, N. P. and Bell, S. D. Classification and Management of Asthma in Childhood. *Am Jour Dis Child* 1922 xxiv 441.
 Rowe, A. L. Bronchial Asthma in Children and Young Adults. *Am Jour Dis Child* 1926, xxxi 51.
 Menagh, Frank R. The Results of Treatment in Bronchial Asthma. *Ann Clin Med*, 1927 v 656.
 Kahn, M. H. Present Status of the Curability of Bronchial Asthma, *Arch Int Med*, 1927 xxxix 621.
³Rackemann, F. M. The Results of the Specific Treatment of Hay Fever, *Jour Immunol* 1926 vi 81.
⁴Pratt, Joseph H. Cardiac Asthma. *Jour Am Med Assn* 1916 lxxviii 809.
⁵Rackemann, F. M. Fatal Asthma. *Boston Med and Surg Jour* 1920 cxvii 531.
⁶Rackemann, F. M. A Clinical Study of 110 Cases of Bronchial Asthma. *Arch Int Med*, 1918, xxi 517.

ESSENTIAL DIFFERENCES IN CHRONIC POLLEN HAY FEVER AND ASTHMA IN CHILDREN AND IN ADULTS*

By I. S. KAHN, M.D. SAN ANTONIO, TEXAS

INTRODUCTION

THE pollens involved in this study were those of the ragweeds, careless weeds, mountain cedar and various grasses. One hundred cases of pollen asthma in children and three hundred cases of pollen asthma in adults comprise the material considered in making deductions. The differences noted in children from the usual manifestations of adult hay fever and asthma were all in cases of combined ragweed and grass sensitiveness. Respiratory system allergic disease of pure animal epithelial emanation, food or other etiology is rare in my community.

The Actual Asthmatic Attack.—Asthmatic attacks in children are said to differ in no way from those of adults. In my experience in the main this is true. In many other instances however the attacks especially in young children are accompanied by high temperature at times reaching 103° or 104° F. which, with the rapid breathing and the appearance of severe illness leads to the diagnosis of pneumonia. Elevated temperature is of course only extremely rarely a part of uncomplicated adult asthma, and careful laboratory

*Read before the American Association for the Study of Allergy, Washington, D. C. May 16, 1927.

and physical examinations so far have revealed no other etiologic factors in these children with febrile asthma. In addition, the prolonged expiratory effort, typical of the adult asthmatic paroxysm, is frequently not seen in young children, especially infants, being replaced by what is apparently an ordinary dyspnea. Generalized rale formation would not bar out a pneumonia, and unless by one means or another definite areas of consolidation are made out, the differential diagnosis may be a matter of doubt. Relief to the difficulty of breathing by the use of epinephrin, of course, solves the problem. That this point is not one of mere academic interest is shown from the fact that in at least half of my cases of children with chronic asthma, a history is given of one or more past pneumonias, in one of my cases four such attacks, the last of which I had the privilege of witnessing. In spite of what seemed a fairly definite unilateral chest percussion dullness, the dyspnea yielded promptly to adrenalin, the temperature disappearing a day or so later. The frequency of the pneumonic histories, three or four attacks in a single young child with a negative chest examination and a negative x-ray picture, and the number of cases in which such a history is given, make it highly probable that many of the pneumonias were actually attacks of febrile asthma. Unfortunately all of my cases of this type so far have been seen where roentgen-ray facilities were not available at the time of the attack. The blood count in these cases may or may not reveal eosinophilia. Leucocytosis is usually present.

That this syndrome does not necessarily indicate a complicating pyogenic infection, is seen from the fact that in the use of sterile pollen extracts for desensitization purposes I have seen several high febrile attacks in children accompany the hay fever and asthma resulting from injudicious overdosage, in one instance, some five or six such attacks.

In a child with previous asthma or a definite asthmatic family history, this point of the presence or absence of actual pneumonia should not and probably would not be overlooked. With the initial asthma paroxysm in a young child, the differential diagnosis may be difficult. In view of the prompt relief available by the use of epinephrin, and the incorrectness of fresh air, outdoor treatment during a pollination season, the importance of this point is obvious. I have seen no harm resulting from a trial diagnostic dose of epinephrin in actual lobar pneumonia in asthmatic children.

Treatment Dosages—For temporary relief in children, somewhat smaller hypodermic doses of epinephrin are required than in adults. I have on several occasions given six or seven minim dosages of this drug without ill effects to infants only a few months old. For permanent relief, however, just as high doses of pollen extracts for desensitization are needed and as well borne. I have in many instances, without unusual difficulty or the slightest harm, reached in children the full dosage of 1 to 2 cc of a standard strength 1:20 ragweed extract, the usual protective dose required in my community. Morphine or opium derivatives are rarely required in the asthma of children.

Preceding Bronchitis—The initial pollen asthma in children is usually or almost invariably preceded by a history of cough or bronchitis of months' or years' duration. I have had occasion to see the condition in children whose

parents were under treatment for hay fever or asthma. There is nothing characteristic in the physical or Roentgen examination of the chest. The onset, recurrence or increase of symptoms during pollination seasons is the only clue in the history. The condition can be recognized by the accompanying vasomotor rhinitis and proved by the immediate alleviation of the condition by epinephrin dosages and its abeyance under an induced pollen free environment, after usual bronchitis regimes and treatments have proved unavailing. The onset of asthma has been prophesied by me and verified in several such cases. As a confirmation I have also produced a most annoying cough without asthma, lasting several days by pollen extract overdosage in desensitizing children suffering from perennial hay fever who had had neither previous accompanying bronchitis nor cough in one case four such attacks. The cough in these cases bears a considerable resemblance to that of pertussis.

Vasomotor Rhinitis—Pollen asthma in children is invariably accompanied or preceded by a definite vasomotor rhinitis. The history in these cases is one of almost constant or frequently recurring so called 'colds' with attendant intermittent or almost unceasing nasal blockage frequently bilateral, but occasionally first on one side and then on the other changing erratically and never permanently unilateral per se. The discharge is watery or mucoid in character. In a case seen in a boy of twelve after his second attack of asthma, the father of the boy a physician stated that the child had not been free from a cold for more than a year. Such a history is typical. In fact, several parents have recalled that the condition has existed since birth. Typical severe seasonal hay fever with persistent sneezing and streaming eyes and noses, is not common in children as it is in adults. In children even ordinary seasonal hay fever is astonishingly mild. Nose rubbing and picking is common in these cases from the itching caused by the lysis of pollen granules on the susceptible nasal mucosa. The ocular palatal and aural irritation of adult hay fever is seldom seen in children. Mouth breathing is exceedingly common. The condition clinically closely resembles typical adenoid disease but is not relieved by adenotonsillectomy. In but few of the childhood asthma cases of this series has this mild vasomotor rhinitis been previously detected though its presence was later invariably confirmed by competent nasal specialists. The diagnosis of this condition can be made out and the pollen etiology established by the rapid betterment or clearing of the condition within a few days under a pollen free environment, and its recurrence through intentional atmospheric or laboratory pollen contact. The appearance of the nasal mucosa is characteristic if seen immediately after such pollen contact. Complicating purulent paranasitis cannot be a common complication of these cases. I have seen only one or two instances.

Chronic Perennial Bronchial Asthma—There is no essential difference in this condition in adults or children. The typical barrel shaped emphysematous chest with hulging sternum often develops in early childhood.

Cutaneous Symptoms—Urticaria and erythema are occasionally seen in adult asthma or a history of a few such attacks in securable. Eczema is exceedingly rare. On the other hand in children angioneurotic edema is very

raie, and urticaria seldom occurs, but present eczema extending over many years, often dating back to infancy, or a history of eczema limited to infancy, is exceedingly common. These cutaneous lesions, both in adults and children, frequently clear under proper pollen treatment without attention to diet or local skin treatment.

Pollen Toxemia in Children—In many children, the physical discomfort and disability are the sole effects of pollen hay fever and asthma, even in severe chronic cases. In addition, however, there appears to be a definite toxic syndrome from constant pollen antigen absorption, to which I have called attention in a recent article.¹ Besides the ordinary vasomotor rhinitis and typical chest findings of asthma or emphysema, these children show marked deficiency of weight, growth and development. The complexion is sallow or of a saffron tinge, the whole appearance greatly resembling that of hereditary syphilis or severe hookworm disease. I have not been able to find anything characteristic in these cases from a laboratory point of view. The most remarkable result of this toxemia is psychic. The mentality is decidedly substandard. Wild spells of anger alternate with long periods of listlessness. These children are extremely cross, irritable and intractable, on the whole in many ways resembling morons. The appetite is poor and capricious. Nocturnal enuresis seems a common accompaniment of the condition. I have seen it persist until after the onset of menstruation. This toxic state seems limited to children, and is seen in hay fever as well as in asthma.

Under a pollen-free environment within a few days, or at the most one or two weeks, with alleviation, but long before the complete elimination of the hay fever and asthma, the toxemia clears. Within even this brief period of time, these children become bright, cheerful and playful. The complexion becomes normal. Appetite returns and weight is rapidly gained. The radical improvement in so short a time has been expressed to me by several mothers as being almost miraculous. The enuresis corrects itself slowly, persisting during desensitization treatment usually as long as any allergic symptoms last, though I saw it clear permanently in one child of eight after only six weeks of such treatment. Shannon² and Piness and Miller³ have called attention to a similar toxemia in allergic conditions in children, not necessarily of pollen origin.

Diagnostic Skin Tests—These were performed intracutaneously in all the cases of this series, and this method of testing was found perfectly practical even in infants, using the back instead of the arm.

It was found as a rule that the typical positive reactions were of smaller size in children than in adults, and as in adults with definite pollen hay fever and asthma, were also at times, especially in young infants, completely negative both immediately and at the end of twenty-four hours. This condition of negative skin tests in pollen asthma was first called attention to by Miss Grothaus and myself in 1925⁴ and 1926.⁵ Peshkin⁶ confirms this finding. The reason for this state of affairs, in the light of our present knowledge, is uncertain. Hypodermic tests with pollen extracts seem to me much more, in fact, perfectly reliable, both in adults and in children. The conjunctival test

with pollen extracts I have not found of much value in either adults or children

In determining the actual symptom etiology of these hay fever or asthmatic children, it was found that the positive skin tests to feathers, animal epithelial emanations and grass root were practically always of clinical significance by actual contact demonstration. Removal of such contact, however, did not affect the clinical hay fever or asthma making additional etiology, of course, obvious.

The frequent positive reactions to one or more foods were of clinical asthma significance curiously enough in not a single case of this series. That is, the use of such specific foods showing positive skin tests would not produce the asthmatic state nor did abstention from such foods abate the asthma. The ingestion of such specific positive skin test foods however was frequently known to produce indigestion or vomiting or had done so in the past. Thus was true also of foods such as milk egg and honey even when the skin tests for such foods were negative. The past production or increase of eczema after certain foods in young children was a common story. Peshkin in a study of 100 asthmatic children about 50 per cent of whom were pollen cases comes to the same conclusion.

The pollen etiology of these cases was demonstrated by the history intra and hypodermic tests the experimental induction of the condition by pollen contact, its abatement or relief under an experimental pollen free environment the details of which have been taken up elsewhere and by the results of pollen desensitization treatment.

Differences in Degrees of Pollen Sensitiveness in Adults and in Children— Practically of course this is difficult to measure. The hypodermic use of pollen extracts up to the point of reaction and symptom production during a period of freedom is the best present method of gauging this point and its measurement in untreated cases over a period of years is obviously not feasible.

While food sensitiveness is ordinarily gradually outgrown the reverse is true of pollen cases living in a heavy constant pollen environment. The histories of such cases living over a period of years in the same homes or communities usually show a definite constant consistent yearly increase in the frequency and severity of asthmatic and hay fever symptoms. Rarely is natal pollen sensitiveness extreme. If such natal extreme sensitiveness were common pollen cases should show severe and frequently recurring symptoms in the first year which is not the case. Though I have seen bronchial asthma as early as the third week of life, and a number of other cases before the end of the first year, usually definite asthmatic symptoms do not occur until the child is at least three or four years of age.

If this initial natal sensitiveness be mild and it apparently is then following the initial pollen hay fever or asthma symptoms in children relief should be practical by reducing pollen dosage by environmental precautions in the home without desensitization treatment or climatic changes. As a matter of fact, the possibilities of such relief are excellent in these early stage chil-

dien I see a number of such cases in young children each year, who, under environmental pollen precautions alone, remain in such manner clear entirely, or with only rare, easily controlled attacks, not over one or two each year

Initial pollen asthma attacks seldom occur in adult life, where there has not been accidentally a change to a heavier pollen environment. In my experience, however, symptom relief by purely household pollen dosage reduction is rarely of avail, certainly not occurring nearly as frequently as with children. On the whole, I feel it safe to say that pollen sensitiveness under constant atmospheric pollen dosage increases with age, especially where atmospheric antigenic pollen is perennial, or practically so. As a matter of fact, resistance to such pollen dosage in an identical environment in potentially hypersensitive cases with positive skin tests, is occasionally tolerated well into adult life before symptom production ensues, even in rare cases until past the age of 60, and in two instances in my records until past 70. One, an asthmatic of 72, with asthma of two years' duration, who, when first seen, had not had a clear day for weeks, secured complete relief in two or three days after removal to a pollen free environment.

CONCLUSIONS

In childhood hay fever and asthma, the following differences are noted from the adult types

1 High temperature occasionally accompanies the asthmatic paroxysm. This, with the attending dyspnea, often leads to the mistaken diagnosis of pneumonia. The required dosages of palliative hypodermic epinephrin treatment are somewhat smaller than in adults. Pollen extract desensitizing doses are identical in adults and in children.

2 A definite bronchitis of months' or years' duration antedates the initial asthma attack.

3 A definite toxemia, with typical physical and mental symptoms, occurs coincidentally in many cases.

4 The accompanying vasomotor rhinitis is much milder than that of adult seasonal hay fever, consisting of more or less constant nasal blockage with mucoid discharge.

5 Complicating purulent sinusitis is rare.

6 The degree of pollen sensitiveness increases with age.

REFERENCES

- ¹Kahn, I. S. Pollen Toxemia in Children, Jour Am Med Assn, Jan 22, 1927, lxxxviii, 241
- ²Shannon, W. R. Neuropathic Manifestations in Infants and Children as a Result of Anaphylactic Reaction to Food Contained in Their Diet, Am Jour Dis Child, July, 1922, xxiv, 89
- ³Piness, George and Miller, Hyman. Allergic Manifestations in Infancy and Childhood, Arch Ped, Sept, 1925, xlii, 9
- ⁴Kahn, I. S., and Grothaus, E. M. Studies in Pollen Sensitiveness, Med Jour and Rec, June 3, 1925, cxxi, 664
- ⁵Kahn, I. S., and Grothaus, E. M. Fallibility of the Intradermal Tests With the Grasses in Chronic Perennial Bronchial Asthma, Med Jour and Rec, March 3, 1926, cxxiii, 290

- ⁶Peshkin, M. Murray Asthma in Children I *Pediatrics*, Am Jour Dis Child, June 1946, xxxi, 763
- ⁷Peshkin, M. Murray Asthma in Children II The Incidence and Significance of Eczema, Urticaria and Angio Neurotic Edema Am Jour Dis Child, Dec., 1946, xxxii, 862
- ⁸Kahn, I. S. and Grothaus, E. M. Efficacy of the Intradermal Tests With the Grasses in Chronic Perennial Bronchial Asthma, *Med Jour and Rec.*, March 3, 1926, cxxix, 90

FURTHER OBSERVATIONS ON THE TREATMENT OF HAY FEVER WITH EPHEDRIN*

By FRED W. GAARDE, M.D., AND CHARLES K. MAXTUM, M.D.†
ROCHESTER, MINN.

THIRTY SIX patients with seasonal hay fever were treated with ephedrin hydrochloride used internally in capsules each containing 25 mg., or applied locally in the form of a nasal spray using a 3 per cent solution. Eleven patients had had preseasonal treatment with only partial relief of symptoms, thirteen patients used both capsules and spray, twelve patients used spray only, and eleven patients used capsules only. Therefore twenty four patients used the drug by mouth and twenty five by local application.

The untoward results varied greatly with different patients. Nervousness and tremor were noticed in thirteen cases, tachycardia or palpitation in five, weakness, faintness or giddiness in four, sleeplessness in two, increased perspiration in three, stimulation in two and nausea in one.

In 70 per cent of the cases the untoward symptoms were absent or mild, while in four cases the nervous symptoms were marked. In this group the nervous symptoms produced by small doses are out of proportion to those obtained with much larger doses in conditions other than hay fever. The following interpretation was suggested: (1) the nervous symptoms bore a distinct relationship to the neurotic tendency of the patient; (2) patients with hay fever are already in a highly nervous state; and (3) the patients treated were all active while other observations have been made on bed patients.

RESULTS OF EPHEDRIN BY MOUTH

Twenty four patients (74 per cent) with hay fever were completely or almost completely relieved for four hours or more; seven (29 per cent) were partially relieved and four (16 per cent) were not relieved or they were unable to tolerate the nervous symptoms.

Ten patients had asthma with hay fever; five were relieved by ephedrin, three were moderately relieved and two were not affected.

*Abstract of paper read before the American Association for the Study of Allergy, Washington, D. C., May 16, 1949.

†Division of Medicine, Mayo Clinic, Rochester, Minn.

Received for publication August 10, 1949.

RESULTS OF EPHEDRIN BY SPRAY 3 PER CENT SOLUTION USED SEVERAL
TIMES A DAY

Sneezing was marked in 12 per cent of the patients. Seven patients (28 per cent) were markedly relieved for several hours, twelve (48 per cent) were partially relieved for several hours or completely relieved for less than an hour, and six patients (24 per cent) were not benefited.

Ephedrin given by mouth in 25 mg. to 60 mg. dosage afforded temporary relief to slightly more than 50 per cent of the patients with autumnal hay fever. An additional 25 per cent were sufficiently relieved to consider its use warranted. Ephedrin given in a 3 per cent solution as a nasal spray is less efficacious and the relief is shorter. Most of the patients, however, felt that it added to their comfort. The best results were obtained when the spray was used early in the paroxysm. The effect of both the local and internal administration seemed to depend upon the severity of the paroxysms, and the good results were obtained in the milder seizures. Eleven patients who obtained only partial relief from preseasonal treatment were able to control their symptoms with the occasional use of ephedrin. The neurotic temperament and nervous state of the patient are important factors in the production of tremor, rapid heart action, and other distressing symptoms produced by the drug.

Although the value of ephedrin is limited and its effect temporary, the observation of fifty-five patients during two seasons warrants the conclusion that the drug should be given a definite place in the symptomatic treatment of autumnal hay fever. It should be emphasized that when good effects are obtained they are temporary and symptomatic.

LABORATORY METHODS

AN APPARATUS FOR RAPID QUANTITATIVE ROUTINE DETERMINATION OF ALBUMIN AND SUGAR IN URINE*

By JAMES J. SHORT, M.D., NEW YORK CITY

THERE are several methods in common use for determining the presence of albumin in urine. With each of these it has been the time honored custom to note roughly the quantity present by the use of such terms as "faint trace," "trace," "moderate amount," "large amount," etc. Naturally the report rendered depends largely upon the individual making the test. What one might report as a "trace" another would call a "faint trace" and so on. For the sugar determination Benedict's qualitative copper solution is widely employed. Slight reductions are very frequently reported as a trace. Quantities above 0.2 per cent are usually determined by some quantitative procedure.

A rather widespread movement has been started by the insurance companies to eliminate the variations resulting from personal factors by instituting routine quantitative methods both for sugar and albumin. Kingsbury, Clark, Williams and Post¹ recently reported a method for the routine quantitative determination of albumin in urine. This is based upon a method devised by Folin and Denis² and consists of the addition of 75 c.c. of 3 per cent sulphosalicylic acid to 25 c.c. of urine using tubes of uniform diameter. After mixing, the tubes are allowed to stand ten minutes and the turbidity produced in each is compared in a Clark lamp³ with standard tubes. The standards range from 5 mg. to 100 mg. of albumin per 100 c.c. and the unknown is reported in these terms.

For routine quantitative sugar determination many laboratories have adopted the Benedict picric acid acetone method⁴. This is a colorimetric method and gives a color with normal urine—the so called normal sugar of urine. One c.c. of urine is employed and the final dilution is up to 25 c.c. in a specially made test tube graduated at the 25 c.c. mark. A comparison is then made with standards in tubes of the same diameter.

The method devised by Sumner⁵ seems preferable to the above for routine work. It is simple, apparently very accurate and seems to give lower and somewhat more consistent results than that of Benedict. The technique is similar and is as follows:

One c.c. of urine is pipetted into the same 25 c.c. graduated tube described above. Three c.c. of the Sumner dimethylglycolic acid reagent are then

*From the Laboratories of the Life Extension Institute, New York.
Received for publication, August 10, 1917.

added, the contents mixed and the tube placed in a boiling water bath for five minutes. It is then cooled, diluted to 25 c c, mixed and compared with the standard tubes of equal diameter in a comparator. Sumner says with reference to the interpretation of results by this method: "Concentrated urines, containing over 0.18 per cent of sugar, or dilute urines, containing over 0.12 per cent of sugar, can be considered abnormal."

It goes without saying that any accurate quantitative method greatly increases the time required for its completion over a qualitative method. Single pipettings require an immense amount of time and make routine quantitative procedures on urine prohibitive, unless the laboratory has unlimited resources of space, personnel and equipment. Apparatus* has therefore been devised

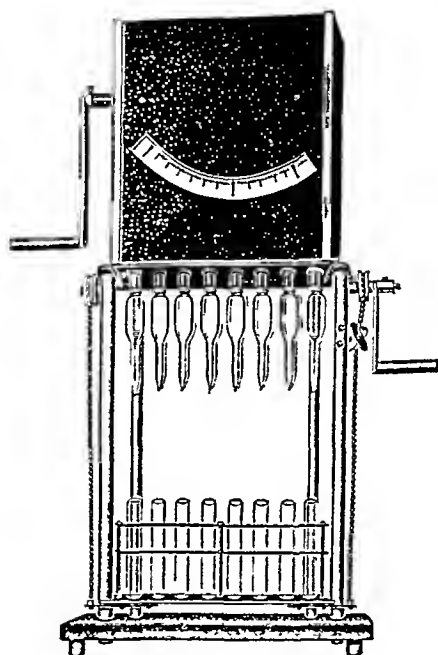


Fig 1 —Multipipette

to make 16 pipettings at one time, with speed, precision and without sacrifice of accuracy. Fig 1 is an illustration of one such apparatus. Two such pieces of apparatus are necessary for the albumin and sugar methods described in this paper—one apparatus to pipette the urine into the albumin and sugar tubes and both to pipette reagents. A specially designed test tube rack constructed of bakelite has been devised for use with this apparatus (Fig 2). Bakelite seems ideal for this purpose as it is tough, does not corrode, withstands great mechanical injury without fracturing and does not soften with boiling.

Briefly, the routine for sugar and albumin with the multipipette is as follows: urines are poured into 15 c c conical centrifuge tubes, placed in two high-powered centrifuges having a total capacity of 16 tubes and centrifuged

*The apparatus described in this paper together with special bakelite racks and modified Clark lamps are manufactured by the Klett Manufacturing Company, 202 East 46th Street, New York, N. Y.

They are then placed in the bakelite 16 holed rack and taken to the first pipetting apparatus. This apparatus has 16 vertically placed record syringes of uniform size connected to glass pipettes as illustrated. The lever at the left of the apparatus returns all 16 plungers simultaneously. A dial records the amount of fluid drawn into the glass pipettes. Three and five tenths c.c. of urine are drawn into each of the 16 pipettes of the first apparatus from each of the 16 centrifuge tubes. The rack containing centrifuge tubes is then withdrawn from the apparatus and the albumin rack containing 16 of the specially graduated albumin tubes* is placed in the apparatus. Two and five tenths c.c. of urine are then discharged into each of the albumin tubes the indicator on the apparatus moving from 35 to 1 c.c. The albumin rack is then withdrawn and the rack containing the 25 c.c. sugar tubes* is placed in the apparatus each tube receiving the remaining 1 c.c. of urine. After this the apparatus is rinsed with distilled water contained in a 2 liter tray which is a part of the apparatus. The rack containing albumin tubes is next taken to the larger apparatus. This is identical with the first apparatus except for greater capacity. Seven and five tenths c.c. of 3 per cent sulphuric acid are drawn into each of the pipettes from a tray holding about 2 liters. (This tray is refilled from time to time from a large reagent bottle



Fig. 1—Bakelite rack

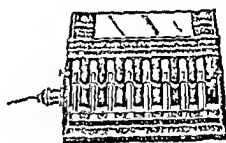


Fig. 2—Modified Clark lamp

and syphon). The albumin rack is placed under the pipettes and each tube receives exactly 7.5 c.c. of the reagent. The rack is allowed to stand for ten minutes and the turbidity then compared with standard tubes having turbidity values equivalent to 5, 10, 20, 30, 40, 50, 75 and 100 mg. of albumin per 100 c.c.* A modified Clark Lamp is illustrated as used in this comparison.

The rack of sugar tubes each containing 1 c.c. of urine as noted above, receives 3 c.c. of the Sumner reagent from the first apparatus. It is then placed in a boiling water bath for five minutes which causes a dark reddish brown color to develop the intensity depending upon the amount of sugar present. After cooling the rack is taken to the larger apparatus. Twenty one c.c. of distilled water are drawn into each of the pipettes and discharged into the sugar tubes making the total volume in each 25 c.c. They are then compared with standard tubes equivalent to 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 per cent of sugar †. Should the amount run higher than 0.5 per cent in the

*The albumin and sugar tubes used in this procedure must be of standard caliber and accurately graduated. Tubes manufactured by Fiske Chemical Company, Shore Road, Cornwall Landing, N. Y., have been found very satisfactory. Permanent standards for the albumin determination are also prepared by this company according to the method described by Fiske.

†The standard tubes are prepared from glucose solution of the following percentages. One c.c. of each is treated in the same manner as the 1 c.c. of urine. As the use of such standards will not change appreciably for several hours. Preliminary experiments in the development of permanent standards indicate that Elmsmark Brown is satisfactory for this purpose—prepared by diluting a concentrated solution to match each of the sugar standards. Dr. Sumner has kindly loaned permanent standards in the form of a 1 per cent sulphuric acid solution in distilled hydrochloric acid which are very satisfactory.

preliminary test, the urine is diluted to fall approximately within the range of standards and the test repeated singly

COMMENT

Actual use of these methods in the analysis of several thousand specimens has given us great confidence in the apparatus and in the procedures. The saving of time is enormous. The accuracy is fully as great as could be obtained by single pipettings. Comparison with previous routine qualitative procedures for sugar and albumin shows that the above outlined procedures can be carried out even more quickly. In the analysis of identical specimens by different workers the albumin and sugar reported have agreed very closely, this takes away the former variations due to the personal element and results in a higher standardization of procedure.

REFERENCES

- ¹Kingsbury, F. B., Clark, C. P., Williams, G., and Post, A. L. The Rapid Determination of Albumin in Urine, *JOUR. LAB. AND CLIN. MED.*, 1926, **11**, 981.
- ²Folin, O., and Denis, W. The Quantitative Determination of Albumin in Urine, *Jour. Biol. Chem.*, 1914, **xxiii**, 273.
- ³Benedict, S. R., and Osterberg, E. A Method for the Determination of Sugar in Normal Urine, *Jour. Biol. Chem.*, 1921, **xviii**, 51.
- ⁴Sumner, J. B. A New Specific Reagent for the Determination of Sugar in Urine, *Jour. Biol. Chem.*, 1925, **lxv**, 393.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

ALBUMINURIA Albuminuria in Children Calvin J. K. Isaacs B. L. and Meyer J.
Jour. Am. Med. Assn. June 12 1926 LXXVI 1821

The significance of albumin in the urine of apparently healthy children is the subject of much discussion.

The authors, after an extended study, offer the following classification:
Benign albuminuria

1 Malnutrition albuminuria frequently associated with anemia, underweight, and systolic basal murmur.

Foci of infection, especially infected tonsils, adenoids, nasal sinuses and carious teeth are common causes of this malnourished condition.

2 Orthostatic albuminuria associated with posture.

3 Idiopathic or 'growth' albuminuria including the terms juvenile, puberty, cyclic, transitory, and intermittent.

Borderline fluids contain 75 to 100 mg. per 100 cc. (0.015 to 0.02 cc. in 2 cc.) This slight increase in the protein content may or may not be significant. Cerebral arteriosclerosis, postepileptic seizure states, encephalitis, head injuries with cerebral edema, treated inactive neurosyphilis, psychoneurotic-like states with toxic foci, alcoholism, multiple sclerosis, degenerative spinal cord conditions and other neurologic conditions at times give this borderline reading. This moderate increase in total protein is of value in differentiating between organic and functional conditions. A positive increase favors an organic diagnosis.

Neurosyphilis (a) Paresis. In this group is found the highest protein content with the exception of the meningitides, xanthochromic fluids and neuronitis cases. The average paretic fluid gives a reading of 200 mg. per 100 cc.

(b) Tabes Dorsalis. The protein estimations in this group have not given high readings. The lowest in treated cases give normal readings, the highest 175 mg. per 100 cc. It is well known that inactive tabes may give completely negative serologic findings. It is rare not to find the protein content increased in tabes and we have found it an aid in a questionable case where the Wassermann was negative.

(c) Cerebrospinal Types. This group consists of the meningovascular cases. The meningitic forms give the highest readings in this group. As a rule the total protein content is not as high as in the paretic group, ranging from 75 mg. to 115 mg. per 100 cc. An average is about 125 mg. per 100 cc.

Cerebral Arteriosclerosis. This group includes neurosyphilitic thromboses, organic dementia, aphasia and so forth, usually old lesions. As a rule the protein content is moderately increased.

Epidemic Encephalitis. In all the positive acute cases a moderate to a marked total protein increase was noted. In the chronic cases the protein content was frequently normal. The pathologic readings averaged 125 mg. per 100 cc. No parallelism was seen between high cell counts and excess protein. Sugar content readings have not been consistent.

Acute Polyneuritis. In the low grade toxic or infectious multiple or mononeuritis cases the protein content has usually been found only moderately increased, about 100 mg. per 100 cc.

(a) Neuronitis or Central Neuritis. In the acute neuronitis cases the readings averaged 150 mg. per 100 cc.

Brain and Spinal Cord Tumor The brain tumor cases almost invariably showed a moderate increase in protein, about twice normal, 100 to 150 mg per 100 cc. One case of syringomyelia with xanthochromic fluid gave an extremely high content, 700 mg per 100 cc, and one case of glioma with rupture by hemorrhage gave a high protein reading. The cord tumors where obstructive give high readings as do other xanthochromic fluids. One cord tumor with evidence of hydrodynamic block gave a lumbar reading of 800 mg per 100 cc and a cisterna magna reading of 100 mg per 100 cc.

Epilepsy These are all cases in which organic factors were suspected but none found except some complicating toxic factors, such as infection, alcohol and so forth. This group showed protein determinations slightly above normal. Following a convulsion the protein content has been more definitely increased.

Cerebral Edema The alcoholics with mental symptoms have all shown increased protein content. The same applies to head injuries showing increased manometric pressure readings. This is a transudative protein excess. The protein readings are about twice normal in these cases.

Meningitis The purulent meningitides give the highest readings. Serous meningitis has consistently shown two to three times normal protein content.

Degenerative Spinal Cord Diseases (a) **Multiple Sclerosis** Only a moderate increase in the protein content was found in these cases. Often the reading is normal. No relation ship between colloidal gold curves and increased protein content was seen.

(b) **Combined Sclerosis** Slight increase in the protein content may be found, but the reading is more often normal.

A reduction in the protein content is an early evidence of serologic improvement.

The method described, though giving slightly higher readings, checks very closely with the Denis Ayer method and is, the authors believe, the more practical clinical procedure.

BILE, BACTERIOLOGY OF Studies in the Bacteriology of Bile, Hansen, S. Hospital stend, Copenhagen, April, 1926, lxix, 289

A total of 414 per cent of samples of bile obtained from autopsies and examined by the author revealed traces of infection.

In some instances this infection is said to have occurred in and to have exhibited an apparent connection with presence of bile, of which it was assumed as the result, while in others the ability of colon bacilli found in bile to live for from six to nine months in the latter was regarded as a probable indication of their viability in such a medium.

Examination of bacterial content of samples of bile collected during operations for gall stones is declared to have demonstrated that 425 per cent of these were infected.

Stones of recent development was a rule encountered together with sterile bile in the gall bladder, a fact which in the opinion of Hansen appears to oppose the view of the importance of infection in formation of gallstones. Old stones were found almost invariably in gall bladders which presented evidences of inflammatory alterations and contained infected bile, an observation which according to Hansen seemed to point to the existence of a secondary infection.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building
Richmond Va

*The Beloved Physician**

THE biography of Sir James Mackenzie written by one who knew his subject from an intimate personal acquaintance by one who possesses the happy faculty of telling his story in a dramatic manner. Sir Mackenzie's life was indeed dramatic.

A biographic sketch of Sir James Mackenzie under review will be found in the editorial pages.

To all physicians who have not thoughtfully and every physician should so classify himself, this work will be delightful reading. The author's frequent use of superlatives and his obvious exaltation fully justify the title of the book.

Principles of Diagnosis and Treatment in Heart Affections†

THE fourth edition of this work by Sir James Mackenzie has been brought to date by his successor at St Andrews James Orr. The work differs from Mackenzie's more elaborate contribution *Disease of the Heart* in that it is written primarily for the practitioner of medicine and provides information which is throughout of an eminently practical nature. As in his other works Mackenzie goes at once to the heart of the matter. Without preliminary tuning up on anatomy embryology histology physiology and the like, he jumps directly into a discussion of heart failure. Indeed it is heart failure which is uppermost in the minds of all victims of heart disease and therefore in the minds of their professional advisors. When will it occur how may it be prevented is this particular case one that is likely to experience heart failure what must be done.

Following this are sections devoted to disorders including coronary myocardial affections prognosis and treatment.

Reports of the St Andrews Institute for Clinical Research Vols 2 and 3‡

THE St Andrews Institute for Clinical Research was recently renamed the James Mackenzie Institute for Clinical Research was established in the little town of St Andrews Fife, for the purpose of studying disease in its inception. The institution was selected in great part because of the permanency of its population. Mackenzie has always been particularly interested in prognosis and insisted that the early pathological changes are recognized the more accurate will be our prognostic deductions. It is known as the motive which inspires not taking with the breath of life making the notes a perennial source of knowledge that can be applied in the practice of medicine.

The Beloved Physician Sir James Mackenzie A Biography by R. McNair Wilson
With a photographure (Cloth Price Pp 216 \$4.00 The Macmillan Company New York 1930)

†Principles of Diagnosis and Treatment in Heart Affections By Sir James Mackenzie
M.D. F.R.C.P. (Ed) and F.R.C.P. (Hon) Director St Andrews Institute for Clinical
Research Consulting Physician to the London Hospital Consulting Physician to H. M. The
King of Scotland and Orr James M.B. Ch.B. Physician to The St Andrews Institute for
Clinical Research Third Edition Cloth Pp 412 Price \$3.50 Humphrey Milford Oxford
University Press American Branch New York

‡Reports of the St Andrews Institute for Clinical Research (Volume II Cloth
Illustrated Pp 190 Price \$3.00) (Volume III Cloth Illustrated Pp 27 Price \$3.00)
Oxford University Press American Branch New York

Volume two of the reports deals chiefly with the principle of the reflex arc and presents a large amount of clinical and experimental evidence bearing upon this hypothesis. This line of thought also runs through volume three, but in both books there are articles on other non-related subjects. In all of them, however, we see clearly the endeavor to study the earliest changes, and if the work of the institute is successful in following the aims of its founder, the magnum opus of the report may not be expected to appear for another twenty or thirty years or more, until after the accumulation, sifting and studying of a tremendous mass of contemporary case records.

This should not and does not interfere with the publication of other investigations, and as we have stated these are devoted especially to the early manifestations. Thus we find articles on the normal infant's chest, clinical studies of influenza, the role of the lymph gland in the absorption of foreign particles and tubercle bacilli (the first defense barrier), the effects of environment on the nervous system of infants and children, clinical manifestations of defective blood supply to voluntary muscles, papers dealing with records obtained in a boarding school for girls, and the like.

*Some Recent Works on Ultraviolet Light**

ULTRAVIOLET light treatment is not new. Its experimental use has extended over more than two decades. We have now reached the stage of its popularization, of its rational and sometimes irrational application to a wide variety of clinical conditions, and of the appearance in the literature of historical and encyclopedic compilations summarizing the observations made to the present time. Among these four in particular may be mentioned as covering the field in sufficiently exhaustive detail.

The work of Francis Howard Humphris¹ does not go into extensive detail but is of interest as presenting a British point of view and as being thoroughly practical from a clinical viewpoint.

The volume by Luckiesh and Paenn² conveys the impression of being written in part at least for the layman and is in spots obviously colored with hyporenthusiasm and claims which, if they are not exaggerated, are certainly not as yet supported by the experimental evidence. There are a few errors of fact, none of great importance, such as might be expected with layman writing on medical subjects. The book, however, is very pleasant reading, is authoritative except where it relates to theories of disease, and may be well recommended.

The third work by Edgar Mayer³ is by far the most exhaustive and authoritative of the three. At the same time it is the most difficult of the three to follow, chiefly because of the extremely large number of excerpts from literature, which we feel at times might have been more systematically grouped. We trust that in his next edition the author will enlarge upon his "chapter summaries," a step which will greatly reduce this disadvantage. The author presents a great deal of original experimental work particularly in the treatment of all forms of tuberculosis. The book is well illustrated. The bibliography is complete.

The three books together with the work by Rollier,⁴ previously reviewed in these columns, will make a quite complete reference library for the student of actinotherapy.*

*For further remarks see editorial pages of this issue.

¹Humphris F. H. Artificial Sunlight. Cloth. Pp 184 with 12 illustrations. Price \$2.75. Oxford University Press American Branch New York.

²Light and Health. A discussion of Light and Other Radiations in Relation to Life and Health. By Luckiesh M. and Paenn A. J. Illustrated. Cloth. Pp 302. Williams and Wilkins 1926.

³Mayer Edgar. Clinical Applications of Sunlight and Artificial Radiation. Cloth, \$10. Pp 550 with illustrations. Williams and Wilkins 1926.

⁴Heliotherapy with Special Consideration of Surgical Tuberculosis by Rollier A. M.D. Cloth. Pp 318. Price \$6.25. Oxford University Press American Branch New York.

The Journal of Laboratory and Clinical Medicine

VOL XII

ST LOUIS MO SEPTEMBER 1927

No 12

Editor in Chief WARREN T VAUGHAN MD

Richmond Va

ASSOCIATE EDITORS

DENNIS E JACKSON MD	CINCINNATI
PAUL G WOOLLEY MD	LOS ANGELES
J J R MACLEOD MB	TORONTO
W C MACCARTY MD	ROCHESTER, MINN
GERALD B WEBB MD	COLORADO SPRINGS
VICTOR C MEYER PhD	CLEVELAND
RUSSELL L HADEN MD	ROSDALE ILL
JOHN A KOLMER MD	PHILADELPHIA
ROBERT A KUDUFF MD	ATLANTIC CITY N J
A PARKER HITCHENS MD	MANILA P I
DEAN LEWIS MD	BALTIMORE
GEORGE R HERRMANN MD	NEW ORLEANS
HAROLD D CAYLOP MD	ROCHESTER MINN

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright 1927 by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis Mo as Second Class Matter

EDITORIALS

The Broader Aspects of Allergy

THE world marvels at those recent advances in physics, chemistry and engineering which have combined to make the present day so remarkable an era in which to live, but it is inclined to overlook the fact that in the ancient profession of medicine, dormant through the middle ages, the last century has produced equally remarkable achievements advances in knowledge many of which have already been applied to the greater comfort and happiness of mankind. Not the least remarkable has been our acquisition of knowledge of those principles underlying the phenomena of immunity and their application in the relief of suffering.

Physiology, since its inception in the master work of Harvey, has been concerned with the vital activities of the living body. Its offshoot, biochemistry, began with the work of Lavoisier and von Leibig at the opening of the nineteenth century. The earlier work fell in the realm of physiologic chemistry with the study of those inanimate organic compounds which are built into vital substance and of the end products which are discarded in the process of living,

together with a study of dead structures which once were living. This was in essence the application of organic chemistry to living structure, and the resulting information was indispensable, but from the nature of the experimental methods little knowledge was acquired of the actual processes of life itself.

For successful study of life, life must not be destroyed at the inception of the investigation. From physiologic chemistry the science of experimental biology has emerged. Here the endeavor is to study the living while still alive. In a crude way the contrast between these two divisions of biochemistry may be likened to the individual snapshots before, during and after a stated activity as contrasted with a cinema reproduction of all the stages and developments in the action.

We may look upon the science of immunology as a rather highly specialized division of the broader field of experimental biology. Immunology has far outgrown its descriptive designation and now includes the study of many phenomena in which immunity as the term is generally understood plays no part. One of these outgrowths from Immunology is that condition of many aspects known as allergy.

Facts are unchanging but our descriptions and interpretations of them are necessarily made in the light of our own past experience and must be altered to accord with more enlightened comprehension. The term immunology is fairly conclusive, and if used in the broader sense of freedom from disease of various sorts whether infectious or not, it may still be applied to the phenomena of clinical allergy. Anaphylaxis, coined by Richet as a term meaning "without protection," is no longer tenable as a descriptive title in view of our present understanding of the phenomena involved. Richet believed that the first injection of a foreign protein destroyed any natural resistance that the animal might possess against the hypothetical poison. The term, however, will remain in the literature, particularly since it is now used to designate a very definite chiefly experimental process characterized by antigen-antibody reaction. Allergy, indicating as it does merely an altered reactivity, is more desirable as a generic term. It commits the user to no single explanatory hypothesis, and hypotheses there are aplenty. It would seem better to continue the use of these terms while realizing their descriptive deficiencies, devoting less energy to lexicographic disputations, particularly since we must realize that the designation of today will not be acceptable tomorrow. These terms and those which are employed along with them should at least suffice until our understanding of the subject has progressed to that stage where we can more confidently give specifically descriptive titles to every phase of the phenomena under study.

Indeed, the student in this field of experimental medicine is not primarily an immunologist, an allergist or an atopist, he is an experimental biologist in the truest sense of the term. He is studying life processes, vital actions and reactions in the living. Few others possess as excellent an opportunity for the study of life itself. The student of allergy is the student of life. True, his chief interest is in what would appear to be an alteration from the

normal vital activity. This we will acknowledge is long as we are discussing allergy as it is observed in man or animal but the evidence would indicate that in their reaction to foreign all agents the *individual cells* of the body are behaving in a strictly normal manner. The abnormality is not within the cells but in their chemical and nutritional environment. Furthermore as is so often the case in other fields of study more can be learned of the normal from a study of deviations from normal than is gained from a study of normality itself.

One of the most important contributions in the origin of life and the development of species has recently been made by an immunologist the groundwork of whose theory is his knowledge of the phenomena of infection and immunity.

The chemistry of allergy is the chemistry of life and no allergist is truly constructive who does not take his way to supplementing his study with experimental investigations of the nature of vital activity. The clinical allergist may reply that he is dealing with human beings on whom he cannot experiment but in truth he is doubly fortunate for his is the opportunity of both studying a naturally occurring manifestation and also correlating this with experimental animal investigation in his laboratory.

We regard as a statement which time will not disprove, that protein is the basis of life. Life does not exist without protein. The student of allergy must be a student of the chemistry of protein and of protein metabolism. The basis of vital activity as we study it today is enzyme action. The allergist must learn of enzymes. There are five fundamental phenomena common to all living substances. First living protoplasm is oxidizable. Deprive it of its state of partial oxidation and it loses its fundamental properties of life. It will no longer conduct an impulse nor will it grow. It will not synthesize or will it move spontaneously. Its respiration ceases and its heat production stops. Second, living substance possesses the ability of synthesizing protein, carbohydrate, fat and other substances. It has the power of growth. This is the chemical process underlying reproduction. This synthesis usually occurs by dehydration. Two molecules are united or condensed

is an alteration in the chemical environment of the cell due to changes in the blood and the lymph. The cell is reacting normally to an altered environment.

A few years back when endocrinology was new and a fad, few diseases to which flesh is heir were omitted by the enthusiast as not being due in part at least to some endocrine disturbance. While the diseases in which allergy appears to be a factor are distinctly limited in number, their character shows such wide divergence that skeptics are inclined to smile. On the contrary it seems almost remarkable that allergy does not manifest itself in an even wider diversification of forms if we realize that we are dealing with a reaction in which many if not all the cells of the body, in widely different locations, are partaking in greater or less degree.

We now discuss anaphylaxis in terms of antigen-antibody reaction and allergy in terms of protein sensitization. The true conception will not be arrived at until we can discuss it in terms of intracellular activity, in terms of the chemistry or the physical chemistry, the energetics of the single cell.

We do not wish to imply that there are not other factors which must be taken into consideration. While the basic allergic reaction lies in the vital activity of the individual cell, the clinical explosion is tempered by modifying activities of different groups of tissues such as nonstriated muscle. This is further exemplified in the work of Manwaring, and of Weil and of Falls' showing the importance of the liver in the production of anaphylactic shock.

It appears not improbable, as more is learned of the normal activity of living protein and particularly of its enzyme reactions, that a reliable means may eventually be developed for the relief of clinical allergy by a single type of therapeutic procedure. Nonspecific desensitization has been widely discussed and often attempted, sometimes with startling success. Results have been inconstant and on the whole inconclusive. Peptone, bacterial vaccines, calcium chloride have each been recommended as nonspecific desensitizers in clinical allergy and have in the occasional case given undoubted relief. Peptone, trypsin, various inorganic salts, foreign proteins, urine, sodium chloride and sodium oleate have produced analogous results in experimental anaphylaxis. The explanations suggested have necessarily been in terms of existing concepts and but to mention them, include such as antianaphylaxis, antisensitization, lowering of the surface tension of the blood, interference with complement action, action on nerve cells, effect upon the colloidal state of the cells and fluids, reducing their irritability, reduction in the speed of reaction between antigen and antibody, alteration of the sensitivity of the reactive mechanism, and tissue inactivation when through exhaustion, drug action or other injury, the sensitized cell cannot respond to the antigen-antibody reaction. Possibly some time, when we have learned more of the chemical processes of life, a simple way may be discovered to modify them so that the reaction of the body cell to an abnormal chemical environment will not be harmful to the body organism as a whole.

The field for possible study and development in allergy, as in other studies of vital activity, appears quite limitless. Methods are already available and new ones are constantly being added. Acquired knowledge in the collateral sciences such as physical chemistry, plant biology, protein and colloidal chem-

istry, protobiology, are all available for application in the further study of allergy. We have but scratched the surface. The mysteries of life remain to be disclosed.

REFERENCES

- ¹Vaughan, Victor C. A Chemical Concept of the Origin and Development of Life, *JOUR. LAB. AND CLIN. MED.* to appear in the October 1925 issue.
²Wells, H. Gideon. The Chemical Aspects of Immunity, The Chemical Catalog Co., New York, 1925.

—W T V

Footprints

PERHAPS it has occurred to you that in his immortal *Psalm of Life* Long fellow has interpolated unnecessary cynicism in intimating that even with the greatest, then impress is made but on the shifting sands and that all too soon the footprints will be lost forever. But Dr. J. C. Merriam, President of the Carnegie Institution, who has made one of the most comprehensive paleontologic studies of footprints, assures us that while the poet was quite in earnest, his meaning was altogether otherwise.

Long before the dawn of human existence when the dinosaur was the lord of creation, these animals were wont to frequent the watering places. The heavy impress of their feet was molded into the moist sand. In some sheltered localities with no human being and few animals of consequence to mar the markings, these footprints remained undisturbed for hundreds, nay thousands of years, until the sand had slowly hardened into stone. Long fellow had seen these dinosaur sandstone footprints in Connecticut and it was their permanence rather than their evanescence which so impressed him.

To the physician few studies are more interesting or more inspiring than that of the footprints left by the great men of medicine. No two stories are exactly alike although many are instructive in their parallelism. Others are absorbing, sometimes tragic in their contrasts.

Not a little pathos surrounds the contrast between Marion Sims and George Harley, two notable contemporaries. Sims, son of a poverty stricken Carolina farmer made out as well as he might with a second rate education, nailed up his shingle in his home town and after the death of his first two patients, found it advisable to change his residence. Imagine his despair. In Montgomery, Alabama, he was no great success. But he had an idea. He thought that he saw a way to the cure of vesicovaginal fistula, that filthy, hopeless penalty of motherhood. Operation after operation was followed by failure after failure and Sims found himself the laughingstock of his Montgomery confrères. But he persisted.

Unable to obtain assistance he built himself a shack in the back yard which he called his hospital and in which he kept his half dozen faithful negro women, all afflicted with the same malady, each of whom would in turn assist when he tried out his most recent theory on her sister in affliction. This was all the operative aid he could muster.

At last he succeeded.

And then he found himself afflicted with an uncontrollable chronic diarrhea which nearly terminated his existence. From his own description we cannot say whether this was pellagra or spine or an amebiasis or other chronic colitis. Probably it was not the diarrhea of pernicious anemia for he lived too long afterwards. But once again he was forced to change his abode, this time for the sake of his own health. He went to New York a poor man with no friends and no supporters. He watched his operation and his special instruments stolen from him by the surgeons of fashion in the great city.

He had the courage of his convictions, however, eventually cheated support, built a women's hospital and before his death found himself the great surgeon of the day, fêted and decorated by the ruling heads of Europe, his amphitheater the surgical mecca of the world.

George Hailey, the wealthy son of an old family from the north of England, accustomed to all manner of luxury, nevertheless a man of brilliant intellect, procured for himself the best and most comprehensive medical education available. Not content he spent several years at the feet of the masters in the most prominent clinics on the continent.

When at last he had satisfied himself with the thoroughness of his preparation he returned to London, opened a consulting office in Hailey Street, the most fashionable section of medical London, established the first chair of physiology in the world in the University of London, and gave promise of becoming one of the greatest physiologists.

And in his thirties he went blind.

Sims and Hailey, the contrast is pathetic.

Edward Livingston Trudeau who had nursed his brother night and day in his hopeless fight against consumption, carefully protecting him in accordance with the doctor's orders, from both the fresh air and sunlight, himself developed the dread disease. He betook himself to the mountains. He did not go there to fight tuberculosis, for in those days there was no fighting. He was a doomed man. His friends begged him not to go, for life in the mountains would but hasten his demise. He went to the mountains because it was there he wished to die, since die he must.

This was in 1873. Dr. Trudeau died in 1915 after having revolutionized the treatment of tuberculosis, brought life and hope to the hopeless and received the homage of the world.

And so the tales of inspiration might go on, were we to allow ourselves this pleasure we would require a volume rather than a few pages. Some tread the path of sorrow and adversity, coming out at last into the glorious sunshine where their footprints, like those of the dinosaur, will endure through history. Others start out under a smiling sun which marks a path both straight and clear but ere their personalities have gained sufficient weight for their footsteps to make an impress deep enough to last, the storm clouds break and disaster overtakes them. Others, less spectacular, just plod along under benign skies and, chiefly because of the straightness of their paths and their avoidance of rambling, they at length reach the pinnacle, there to plant the standard, symbolic of their contribution, great or small, toward the well fare and advancement of mankind.

But we cannot resist the temptation to write further of one who, at the apogee of success considered himself in one respect at least a failure. So far the world has not granted him his self condemnation. Posterity will be the better judge.

James MacKenzie considered himself a plodder. From his own description his preliminary education was none too fortuitous. He had no patience with an educational system whose chief or only criterion of ability or scholarship was a capacity for memorizing. He would have preferred a system designed more to develop the faculties of reason. After a medical school career which seems to have been characterized neither by brilliancy nor torpidity he entered general practice. As with Sims a patient died. This was the turning point and the starting point of MacKenzie's career.

A pregnant woman with heart disease died suddenly and unexpectedly during labor. Why could he not have foretold the danger in this case? MacKenzie went to his books and to the learned men of the time but an answer was not forthcoming. Plenty of information could be got on the character of the heart lesion and of heart lesions in general but as to prognosis and as to what lesions shall be considered of serious import and which of little consequence there was nothing to be told. MacKenzie set himself the task of finding out and through years of painstaking study and carefully kept clinical records, following his patients from the earliest inception of a disease, through the years to its final outcome, he gradually developed the modern science of clinical cardiology. He revolutionized our concept of heart disease.

He like George Harley moved into Harley Street but late in his career after his reputation had been well established.

MacKenzie went from little Bunkley to Great London not that he might be a specialist, for he detested the term but that through closer contact he might convince the reactionaries in medicine of the necessity for reorganization of their viewpoints on heart disease. Here he found himself coerced, against his will and in spite of his protestations into the position of a heart specialist. In London he built a great new school of cardiology but he despised the distinction and implored his students to return to general practice as the only place in which chronic disease may be studied in its incipency and followed to its termination. It was in the milieu of general practice that he confidently expected the great clinical advances in the medicine of the future. So we see an old man disheartened by the failure of his pupils to consider seriously his entreaties determined that if none other will go, he at least must follow the light moving his home once again this time to the little town of St Andrews in Scotland, there to take up again the study of disease from the viewpoint of the general practitioner.

There the great Sir James MacKenzie passed most of his later days interested and active to the end in the work of the St Andrews' Institute for Clinical Research which he had founded for the perpetuation of those viewpoints and ideas which he had striven so hard to make his.

In his work, Sir James developed a machine. Others bestowed upon it his name. This was the MacKenzie Ink Polygraph. It brought him great renown. The satisfaction of recognition was, however deeply tempered by

the knowledge that in the minds of many it was the instrument that was worshiped and not the increasing knowledge made possible through its use. In nearly all his later contributions he did his utmost to counteract this tendency by stressing the desirability of using precision instruments only so that one may be better equipped to diagnose and treat diseases of the heart without them.

What will posterity have to say of Sir James MacKenzie? Will he, like Boerhaave and Osler, go down as one of the clinicians of his time, a prolific writer, a man to whom the world made pilgrimage, but who has contributed no truly great outstanding stepping stone to the progress of medicine? The principle of the reflex arc still remains in the balance. The exhortation to study disease in its law is evangelistic but dies with the man and is perpetuated only in as far as his personality is built into his writings. The prominence and influence of the MacKenzie Institute will be dependent entirely upon the greatness and the vision of the minds that will inhabit it.

Nevertheless we venture to believe that MacKenzie's advancement of our knowledge of cardiology, quite aside from his work with the polygraph, will assure the passage of his name through many generations of the followers of Hippocrates.

—W T V

INDEX TO VOLUME XII

AUTHORS INDEX

In this index following the author's name the title of the subject is given as it appeared in the Journal. Editorials are also included in the list and are indicated by (E)

A

- ALLEY, R C (See Haskell Alley and Prillman), 313
 ALTNOW, HUO O AND CAREY JAMES B A case of polythemia vera treated with phenylhydrazin hydrochloride with special reference to changes in blood morphology, 597
 ARKUSH, ALBERT (See Proescher Arkush and Krueger) 908
 ARNOLD LLOYD AND WEISS EMIL Prophylactic and therapeutic possibilities of the Twort d'Herelle's bacteriophage, 20
 ARONSON, JOSEPH D The standardization of tuberculin 169

B

- BALDWIN LOUIS C B Routine skin tests in chronic nephritis 1
 BALVEAT, RAY M Factors which determine the pollen content of the air 1151
 BARTLETT WALTER M The effect of hypodermic insulin on the fasting blood sugar in normal and diabetic subjects in relation to percentage normal weight 115
 BARTON R B The endotheloid cell in acute leucemia 855
 BAZETT, H C AND MCGLOTH B A portable thermoelectric apparatus for the determination of surface and tissue temperatures 913
 BECKFR, S WILLIAM The anticomplementary reaction of blood serum to the Kolmer complement fixation test controlled by the reaction to the Kahn precipitation test, 26.
 — The sugar and chloride content of the cerebrospinal fluid with special reference to neurosyphilis 43
 BENDIXEN P A, AND LAMB F H Malignant tumors of the adrenal in children 130
 BLACK J H The oral administration of pollen 1156
 BLALOCK ALFRED A rubber mask for determination of oxygen consumption of the dog 378
 BOND W R (See Irving and Bond) 882
 BROWN GRAPHER TYLER Eosinophilia in asthma hay fever, and allied conditions 1145
 BUCKER HENRY A JR AND MEYERS SIDNEY Blood groups in general paralysis 415

- BURBANK REYNOLD (See Hadjopoulos and Burbank) 913, 979
 BUTLER C S AND PETERSON F Treponematoses as seen in the rural population of Haiti, 670
 BYRD THOMAS LUTHER Studies of quantitative blood sugar estimation 609

C

- CATHOUN J A (See Widdell and Cathoun), 991
 CANTAROW A (See Caven and Cantarow) 73
 CAREY JAMES B (See Altnow and Carey) 597
 CAVE: W R, AND CANTAROW A A method for the determination of calcium in whole blood 70
 CAYTOR HAROLD D A new method of examining fresh tissue (E), 103,
 CHANDLER JOSEPH The determination of fibrin in blood plasma 1092
 COCA ARTHUR F Studies in hypersensitivity 1135
 COHEN SEYMOUR J Studies in local anesthesias VI 983
 COOKE EDWARD F Welcome to Dallas, 661
 CRAVER LLOYD E Metastasis to thyroid gland from endothelial nevioma of bone rapid regression resulting from roentgen ray treatment 878
 CROCKER FRED L (See Jones and Crocker) 482

D

- DAVENPORT C K (See Gilbert and Davenport), 914
 DAVENPORT H A A note on the Koch and McMeekin method for the determination of nitrogen with special reference to the nonprotein nitrogen of blood and urine 286
 DAWSON W T A note on chloroform anesthesia of dogs, 139
 DE ENS, FLOYD Normal blood counts in pigeons, 437
 DOCK, WAR (See Hartman and Dock), 430
 DUNN MARTIN A colorimetric method for the determination of iron and hemoglobin in the blood 917
 — A modified digestion acid for nonprotein nitrogen determination, 387
 DURANT R R An improvised incubator 1011

E

- EBEL, ELSA (*See* Thompson and Ebel), 1087
- ELVEHJEM, C A, AND WADDELL, J A A standard method of recording the hemoglobin content of blood, 889
- ERLANGER, JOSEPH, AND MEEK, W J An adjustable sphygmoscope for the recording sphygmomanometer, 172
- ERVIN, DWIGHT Acetone body formation and the chemical affinity of oxygen for carbohydrate and fatty acid, 1059
- A contribution to the nature of diabetes A mathematical derivation of the blood glucose curve, 318
- EVERETT, MARK R, AND HART, M O The determination of sugar in normal urine, 579

F

- FEDER, J M Intestinal amebiasis from the pathologist's standpoint as related to the clinical picture, 451
- FLEMSTER, ROY F A variable filter for the microscope, 1116
- FERRY, N S, AND FISHER, L W The rôle of the washings of Streptococcus scarlatinae in scarlet fever, 274
- FISHER, L W (*See* Ferry and Fisher), 274
- FORMAN, JONATHAN The sedimentation rate of erythrocytes, the corpuscle volume, and the reticulocyte index, 373
- FOX, HERBERT, AND LEAMAN, WILLIAM G Observation on blood cultures with a special reference to the quantity of the blood used, 145
- FULLER, HENRY C, AND HUNTER, OSCAR B Isopropyl alcohol—an investigation of its physiologic properties, 326

G

- GAARDE, FRED W, AND MAYTUM, CHARLES K Further observations in the treatment of hay fever with ephedrin, 1203
- GIBBS, O S Drop recorder, 686
- GIBSON, H V A method for capillary blood sugar, 906
- GILBERT, RUTH, AND DAVENPORT, C K Failure of the mouse test to demonstrate the presence of type I pneumococcus in sputum An unusual instance, 944
- , AND STEWART, CONSTANCE Corynebacterium ulcerans A pathogenic microorganism resembling C diphtheriae, 756
- GIORDANO, ALFRED S The diagnostic value of the sugar content in the cerebrospinal fluid, 858
- GRADWOHL, R B H Description of a new hydrogen ion colorimeter, 694
- GRAHAM, GEORGE S, AND MCCARTY, SARAH H Notes on sickle cell anemia, 536
- GRAY, HORACE Diabetic gangrene treated by insulin, 472

H

- HANG, H B, AND BOND, W R Some notes on glycol, glycol chlorotone anessthesia, 882
- HADFN, RUSSELL L Progress in the treatment of pernicious anemia, (E), 722
- The elective localization of bacteria in heart and vascular disease, 31
- HARDENBERGH, JOHN G Epidemic lymphadenitis with formation of abscess in guinea pigs due to infection with hemolytic streptococcus, 119
- HADJOPOULOS, L G, AND BURBANK, REGINALD The origin and nature of the Wassermann antigen, 973
- AND — The duality of the antigenic nature of erythrocytes, 979
- HALL, W W The Van Den Bergh reaction for serum bilirubin with notes on interpretation and technique, 529
- HART, M O (*See* Everett and Hart), 579
- HARTMAN, F W (*See* Jackson and Hartman), 442
- HARTMAN, HANS, AND DOCK, WM The use of cholin in paralytic ileus, 430
- HASKELL, CHAS C, ALLEY, R C, AND PRILLAMAN, P E Esanguination transfusion in treatment of phenol poisoning, 313
- HASKINS, HOWARD DAVIS (*See* Holbrook and Haskins), 10
- HACKER, F A The treatment of one hundred five cases of acid intoxication with buffer solutions, 467
- HEIN, GORDON E Cedar oil as an aid in finding parasitic ova in feces, 1117
- HENRY, LUCY DELL, AND MARSHALL, M S The stability of carbohydrate mediums, 474
- HERRMANN, GEORGE R Some clinical tests for the estimation of circulatory respiratory functional efficiency, (E), 828
- HERSOHN, W W (*See* Kilduffe and Hersohn), 810, 946, 988
- HILLKOWITZ, PHILIP The integration of hospital laboratory work, 666
- HOLBROOK, WILLIAM PAUL, AND HASKINS, HOWARD DAVIS Blood uric acid in nephritis, 10
- HOLLISTER, GUY L (*See* Stier and Hollister), 1139
- HOLMAN, W L, AND KROCK, F M The effect of paraffin and oily substances upon filter candles, 158
- HUEPER, WILHELM Mesenteric enterocystoma, 427
- HUNTER, OSCAR B (*See* Fuller and Hunter), 326

I

- ISHAM, ROBERT M The use of isopropyl alcohol in the preparation of Wassermann antigens, 52
- IVY, A C (*See* McEnery, Meyer, and Ivy), 349, 362

J

- JACKSON, D E A new type of motor driven long paper kymograph 1 0
 — A universal artificial respiration and closed anesthesia machine 998
 — Ephedrin (E) 624
 JACKSON, EDNA AND HARTMAN F W Experimental bacillus pyocyanus keratitis 442
 JACOBS MAURICE S (See Steinfeld and Jacobs) 850
 JONES RODNEY Oxalic Acid A good white cell diluting fluid 614
 JONES WALTER CLINTON AND CROCKER FRED L Leucocytic indices of body resistance with report of a new index 482

K

- KAHN, I S Essential difficulties in chronic pollen hay fever and asthma in children and in adults 119
 KAHN MAX Effect of Bacillus aerophilus on intestinal putrefaction and indican output 226
 KATAYAMA ICHIRO Studies in blood glycolysis 239
 KENOE ROBERT A On the toxicity of tetraethyl lead and inorganic lead salts 554
 KILLOGG WILFRED H AND WELLS L AMY The influence of magnesium salts on amboceptor and complement titrations 153
 KILDUFFE ROBERT A Cults and their relation to medical practice (E) 1033
 — A note upon the preservation of sheep cells for use in complement fixation tests 255
 — The epidemiology of pneumonia (E) 309
 — Details (E) 311
 — Liver function test (E) 3
 — The chemotherapy of streptococcemia 572
 — The menace of the slightly positive Wassermann 627
 — The etiology of granuloma inguinale (E) 929
 — Laboratory examinations—not laboratory test (E) 1133
 — AND HENSON W W The preparation of colloidal gold solution 810
 — AND — A study of the micro Kahn test in syphilis A report of 2100 reactions 946
 — AND — A note upon the bacteriology of excised tonsils 988
 KILLIAN JOHN A (See Shattuck Killian and Preston) 802
 KLEIMANN HANS On nephelometry 629
 KLINE CORDON M The demonstration of bacteriophage in old stock cultures 1074
 KLINE B S AND YOUNG A M A microscopic slide precipitation test for syphilis 477

- KOBER PHILIP ADOLPH The preparation of potassium and sodium tetrabismuth tartrates 962
 KRASNOW FRANCES (See Rosen and Krasnow) 157
 KROCK F M (See Holman and Krock) 158
 KRUEGER ALBERT (See Proescher Arkush and Krueger) 908
 KRUEGER A P (See Proescher Ryan and Krueger) 57

L

- LAMB F H (See Bendixon and Lamb) 130
 LEAMAN WILLIAM G (See Fox and Leaman) 145
 LEAS R D Reducing substances in the urine 1
 LEIBOFF S L A note on the measurement of blood for chemical examination 911
 — A rapid method for the determination of chlorides in blood or urine 702
 LEVINE VICTOR E AND MAGIERA ESTELLE A Studies in toxicologic chemistry 773
 LEWIS W B The glucose tolerance test, 380
 LIPPINCOTT LEON S Hemoglobin and erythrocytes in the south 679
 LOWY OTTO Chemical results with pathogen 460

M

- MACLEOD J J R New views on carbohydrate metabolism (E) 719
 MAGIERA ESTELLE A (See Levine and Magiera) 773
 MANHEIMS PERRY J AND McGRATH IRENE S A simple blood culture technique 607
 MARSHALL M S (See Henry and Marshall) 474
 MAYTUM CHARLES K (See Gaarde and Maytum) 1203
 McCARTY SARAH H (See Graham and McCarty) 536
 MCCOY ELIZABETH F (See Wright and McCoy) 790
 McENERY E T MEYER JACOB AND IVY A C Studies on nephritis II Gastric secretion in nephritis 362
 — AND — Studies on nephritis I Physiologic and anatomic changes following temporary ischemia of the kidneys 349
 MCGLONE B (See Bazett and McGlone) 913
 McGRATH IRENE S (See Manheims and McGrath) 607
 MCGILVER H A The pharmacology of iron and aluminum in relation to therapeutic uses 790
 McJUNKIN F A The large mononuclear and the transitional leucocyte of human blood 71
 McMINN H E (See Piness and McMinn) 1164

- MEER, W. I. (See Erlanger and Meek), 172
- MEINCKE, ERNST (Translated by Mollmann, A.), Flocculation test in the serology of syphilis, 891
- MEYER, JACOB (See McEmery, Meyer and Ivy), 349, 362
- MEYERS, SIDNEY (See Bunker and Meyers), 415
- MOISE, THEODORE S. (See Sooy and Moise), 1113
- MOLLMANN, A. (See Meincke), 891
- MOORE, ANNA C. A note on the method of diluting antigens for use in the complement fixation test for syphilis, 594
- MOORE, ROBERT A. (See Scott and Moore), 461
- (See Spohn and Moore), 435
- MYERS, VICTOR C. Alkalosis (F), 101

N

- NICHOLSON, DANIEL A combined diluting and staining fluid for differential leucocyte counts in the counting chamber, 548
- NIXON, CHARLES E. (See Rusk and Nixon), 644
- NIXON CHARLES E., AND OLIVER, JEAN Early infantile progressive muscular atrophy (Werding Hoffmann) A chemical and pathologic study of two cases, 837

O

- OGAWA, ISAMU A note upon complement fixation in tuberculosis, 563
- O'KEEFE, EDWARD SCOTT Influence of the capillary circulation upon certain allergic conditions, 1149
- OLIVER, JEAN (See Nixon and Oliver), 837
- ORENT, ELSA R. Sugar in blood, 432
- OSGOOD, EDWIN E. Tables for calculation of color index volume index and saturation index based on recently determined standards, 899
- OSTERBERG, ARNOLD E., AND STRUNK, JOY A comparison of the Folin Wu and new Benedict methods for the estimation of blood sugar, 275

P

- PATTERSON, T. L. Gastric movements in the pigeon with economy of animal material. Comparative studies, 1003
- PEARCY, J. FRANK, AND WEAVER, M. M. Barbituric acid derivatives as anesthetics and methods for administration, 1071
- PENDLETON, WALTER R. Methods for preventing the agglutination of blood by glucose solutions, 369
- PETERSON, E. (See Butler and Peterson), 670
- PIATETZKY, A. (See Short and Piatetzky), 1098

- PICKARD, RAWSON J. The pathogenecity of the small races of the "Amoeba dysenteriae," 743
- The amoeba *conneximma laffeurii*, its appearance and clinical importance, 1037
- PINTSS, GEORGE, AND McMINN, H. E. The role of the structural features of pollen grains in identifying the most important hay fever plants of California, 1164
- PLASS, E. D., AND ROURKE, M. D. The effect of venous stasis on the proteins of blood plasma and on the rate of sedimentation of the red blood corpuscles, 735
- POLOWE, DAVID The blood urine density index, 1100
- PORKO, THOMAS J. Rapid mixing flasks for the preparation of the standard antigen dilution used in the Kahn precipitation test, 132
- PRESTON, MARJORIE (See Shattuck, Kilbrin, and Preston), 802
- PRILLMAN, P. E. (See Hiskell, Alley, and Prillman), 313
- PROESCHER, FREDERICK ARKUSH, ALBERT, AND KRUEGER, ALBERT Methods for making a stable emulsified syphilitic antigen, 908
- PROESCHER, F., RYAN, F., AND KRUEGER, A. P. Report of a case of eosinophilic granuloma with autopsy findings, 57

R

- RACKEFMANN, FRANCIS M. Studies in Asthma, A clinical survey of 1074 patients with asthma followed for two years, 1195
- RAMSDALE, SUSAN GRIFFITH A note on the complement fixation reaction in rat tipollen serum, 259
- RICHTER, CURT P., AND WANG, GING H. New apparatus for measuring the spontaneous motility of animals, 289
- ROCKWOOD, REED, AND SZCZYPANSKI, ADAM Modification of the icterus index, 813
- ROHDENBURG, G. L. An analysis of 500 tumors of the ovary, 211
- ROOS, C. On the cultural characteristics of *Haetobacillus acidophilus*, 1053
- ROSEN, ISIDORE, AND KRASNOW, FRANCES A note on the calcium content of the serum of normal adults, 157
- ROSS, S. GORDON A study of bile secretion from a case of biliary fistula, 1067
- ROSS, VICTOR Immunization of rats against pneumococcus by feeding the acid killed germs and the influence of the age of the animal thereon, 566
- ROHM, PAUL Oxygen therapy A method of administration and apparatus, 388
- ROURKE, M. D. (See Plass and Rourke), 735

- ROWF ALLAN WINTER. A new disk for use in basal metabolism determinations, 590
- Note on the quantitative determination of arsenic in organic material 150
- RUCKER JAMES B. JR. A simplification not a modification of the Kolmer test, 1109
- RUEDIGER E. H. Comparison between the Kahn flocculation test the Kolmer Wassermann test and the Ruediger Wassermann test 269
- RUSE GLANVILLE I. AND DIXON CHARLES E. Diffuse cortical sclerosis: a clinical and pathologic report of two cases 644
- RYAN E. (See Proescher Ryan and Krueger) 57
- S
- SAETHRE, H. (See Thjotta and Saethre) 762
- SALLE A. J. A simplified method for the preparation of special liquid blood media by filtration 1009
- SANDERSON FVRETT S. Spontaneous death in a rabbit with the isolation of a type IV pneumococcus 106
- SANFORD, ARTHUR H. A photographic method of counting blood cells 456
- SCHWENTKER, FRANCIS F. A simpler method for the preparation of potassium pyrogallate solution for metabolic rate determinations 247
- SCOTT, FRANK, AND MOORE ROBERT A. Ochronosis 461
- SHATTUCK HOWARD F. MILLIAN JOHN A. AND PRESTON MARJORIE. A comparison of the quantitative methods for the bilirubin of the blood 80
- SHAW FREDERICK W. An infection of man probably due to salmonella suspected, 141
- A moulter from the respiratory tract 968
- SHAWKER MAX. A convenient method of collecting small amounts of serum 184
- SHORT JAMES J. An apparatus for rapid quantitative routine determination of albumin and sugar in urine 1205
- AND PRATETSI A. A Note on urine preservatives 1098
- SILVETTE, HERBERT. A chart for reading the color index of blood 1108
- A device for the dilution of antigen in the Kahn precipitation test 1096
- SLEPPER FRANCIS HARPER. (See Walker and Slepper) 1048
- SOMOGYI MICHAEL. Note on the tungstic acid precipitation of blood proteins 500
- SOOY, J. W. AND MOISE THEODORE S. A method for the determination of the coagulation time and retraction time of the blood 1113
- SPOHR, CARL L. AND MOORE ROBERT A. A study of the pigment in Addison's disease 438
- STARK, MARIO E. The chloride content of canned smetkrant 561
- STASIA I. The influence of diet on the physiologic assay of insulin 256
- STEINFELD EDWARD AND JACOBS MAURICE S. A study of the toxic filtrates of anhemolytic streptococci recovered from patients with rheumatic fever 850
- STEWART CONSTANCE. (See Gilbert and Stewart) 756
- STEWART ZELLA WHITE. Treatment of rheumatic patients affected by protein of the epidermal group 1179
- STIER ROBERT F. E. AND HOLLISTER GUY L. A comparative study of pollen antigens as determined by the skin reaction 1139
- STONER WILLIAM H. Notes on basal metabolism. IX. Simplified calculation for gasometer gas analysis method 884
- STRUNK JOY. (See Osterberg and Strunk) 248
- SWAN MARY H. Three phases of Wassermann technique. Associated considerations: a comparison of antigens, and a method of titrating and estimating positivity of a serum 965
- SWOJINSKI ADAM. (See Rockwood and Swojinski) 813
- T
- TAJIMA SHIRO. Japanese medical education (E) 896
- THJOTTA TH. AND SAETHRE H. Observations on the relationship of the Wassermann reaction cells and globulin content and of colloidal gold precipitation reaction of spinal fluid in syphilitics 762
- THOMPSON LAWRENCE D. AND EBEL ELSA. A comparison of 10 000 Wassermann and Kahn tests run in parallel 1087
- THURMAN L. A. The difficulties and value of frozen section methods 492
- U
- ULFR LEON. Clinical investigations in allergy 119
- V
- VAN ALLEN C. M. Volume measurement of blood platelets 282
- VANHAAN WARREN T. The functional pathology of nephritis (F) 204
- Sclerosis of the pulmonary artery (E) 411
- The reviews (E) 414
- Some recent contributions on the treatment of pneumonia (E) 931
- The resections of the omentum 1028

- VAUGHAN, WARREN T The broader aspects of allergy, (*E*), 1213
 — Footprints, (*E*), 1217
 VISHFR, JOHN W Spirochetal bronchitis
 Report of a case successfully treated with arsphenamine, 55

W

- WADDELL, J A The action of pilocarpin on the rat's pupil, 232
 — AND CALHOUN, J A The action of indol and skatol on the heart, 991
 WADDELL, J (*See* Elvehjem and Waddell), 889
 WALKER, BURNHAM SARLE, AND SLEEPER, FRANCIS HARPER Tryptophane reactions in the spinal fluid, 1048
 WANG, GING H (*See* Richter and Wang), 289
 WATSON, E M A method for estimating blood pressure in rabbits, 706
 WEAVER, M M (*See* Perrey and Weaver), 1071

- WEBB, GERALD B Ring and Flocculation tests in tuberculosis, (*E*), 1023
 WEISS, EMIL (*See* Arnold and Weiss), 20
 — The behavior of bacteriophage in sugar media, 937
 WELLS, L AMY (*See* Kellogg and Wells), 153
 WRIGHT, WILLIAM H, AND MCCOY, ELIZABETH F An accessory to the isolation of single bacterial cells, 795

Y

- YOUNG, A M (*See* Kline and Young), 477
 YOUNGBERG, GUY E The organic phosphorus of the cerebrospinal fluid, 845

Z

- ZIEGLER, W H Sodium thiosulphate and calcium salts in prevention of the sequelae of illuminating gas poisoning, 109

SUBJECT INDEX

Abdominal puncture in diagnosis of acute intraperitoneal disease 403
 Abstracts, 78 185, 293 393 501 615 711 814, 921 1013 1119, 1209
 Acetone body formation and the chemical affinity of oxygen for carbohydrate and fatty acid 1059
 insoluble antigen 53
 Acid blood uric in nephritis 10
 intoxication treatment of with buffer solutions 407
 Acidosis in the toxemia of pregnancy 81
 Acids and base, concentration of in normal plasma 393
 Acute arteritis case of 41
 auricular fibrillation case report of 33 38
 leucemia endotheloid cell in 8
 myocarditis case report of 33
 phlebitis and myocarditis case report of 37
 Addison's disease pigment in study of 438
 Adenocarcinoma arising from cystadenoma 219
 Adrenal in children malignant tumors of 130
 Agglutinin agglutinin formula study of in ninety one cases 415
 Agranulocytosis 814
 Air, pollen content of factors which determine 1151
 Albumin and sugar in urine apparatus for rapid quantitative routine determination of 1205
 quantitative determination of by Timm 1020
 Albuminuria in children 1203
 Alcohol excretion of in the urine as a guide to alcohol intoxication 296
 isopropyl, preparation of Wassermann antigens in 52
 Alcoholic intoxication excretion of alcohol in the urine as a guide to 296
 extracts simple 53
 Alkalosis 104
 Allergic conditions influence of capillary circulation upon certain 1149
 rhinitis 1162
 Allergy, broader aspects of 1213
 clinical investigations in 11, 10
 eosinophilia in 1146
 Alopecia syphilitic its relation to neurosyphilis 295 519
 Amboceptor titration 154
Amoeba cuniculiformis *laforcei* its appearance and clinical importance 1037
 of dysentery pathogenesis of the small races of, 743
 Amoebae, detection of in cases of chronic systemic amoebic infection 1015
 Amoebic infection 1015

American Society of Chemical Pathologists, 725
 the fifth convention, 708
 Acidity diagnostic value of 714
 Anaerobes spore forming blood agar plate for 295
 Anaerobic infection, 924
 Anaphylaxis relation of to immunity 505
 Anemia familial pernicious 1014
 pernicious blood sugar in 190
 blood transfusion in 299
 etiology and treatment of 921
 high color indices in 714
 progress in treatment of 722
 treatment of by special diet 819
 relation of primary and secondary to vitamin A deficiency 923
 relationship between renal insufficiency and 512
 sickle cell 536
 Anesthesia chloroform of dogs 139
 glycol chloroform 882
 local studies in 983
 machine universal artificial respiration and closed 998
 Anesthetics barbituric acid derivatives as and methods for administration, 1071
 Angina pectoris myocarditis with case report of 39
 Antihemolytic streptococci toxic filtrates of recovered from patients with rheumatic fever 850
 Animal material economy of gastric movements in the pigeon with 1003
 Anthrax bacilli immunization of sheep by means of 926
 Anticomplementary reaction of blood serum to the Kolmer complement fixation test controlled by the reaction to the Kahn precipitation test 265
 Antigen acetone insoluble 53
 in the Kahn precipitation test, device for the dilution of 1096
 syphilitic methods for making a stable emulsified 308
 water soluble purification of 504
 Wassermann origin and nature of the 973
 Antigene nature of erythrocytes, duality of 979
 value of the toxin antitoxin precipitate of Ramon 503
 Antigens method of diluting for use in the complement fixation test for syphilis 594
 Wassermann isopropyl alcohol in preparation of 52
 Antipneumococcus serum and antibody solution specific precipitates obtained from by the soluble specific substances of pneumococci, 303
 Antipollen serum complement fixation reaction in 2, 9

- Apparatus for measuring the spontaneous motility of animals, 289
for rapid quantitative routine determination of albumin and sugar in urine, 1205
for the isolation of single bacterial cells, an accessory to the chambers, 795
thermoelectric, a portable, for determination of surface and tissue temperatures, 913
- Appendix, bilharzia infection in an apparently normal, 189
- Arsenic in organic material, quantitative determination of, 150
- Arsphenamine, spirochetal bronchitis, successfully treated with, 55
- Ateritis, acute, case of, 41
pulmonary, 409
recurrent acute phlebitis and, case of, 40
- Aspergilli, 521
- Asthma, and hay fever, 825
differences in, in children and adults, 1197
eosinophils in, 1145
extrinsic, 1187
intrinsic, 1191
studies in, clinical survey of 1074 patients followed for two years, 1185
- Asthmatic patients affected by protom of the epidermal group, 1179
- Atopy, influence of heredity on, 1135
- Auricular fibrillation, acute, case report of, 33, 38
endocarditis, case report of, 34
- B
- Bacillus acidophilus, effect of, on intestinal putrefaction and indium output, 226
Koch, staining methods for, 514
procyaneus keratitis, experimental, 442
tuberculosis, skin reactions with filtrate of Koch strain, 513
- B bifidus, character and isolation from the intestine of infants, 99
- B diptheria, culture medium for, 101
- B proteins, nature of the soluble proteolytic enzyme of, 393
- Bacteria, flagella, abnormal, in cultures, their resemblance to spirochetes, 511
food poisoning, differentiation of, 90
from infected teeth, localization of, 32
in heart and vascular disease, elective localization of, 31
- Bacterial flagella, method for the staining of, 404
flora of cancer of the breast, 616
growth, inhibition, by some amino acids and its bearing on the use of tryptic digests as culture media, 504
spores, studies upon, 501
- Bacteriologic examination of urine for Koch's bacillus, 186
investigation of material containing proteins 510
- Bacteriology and applied immunology for nurses, 408
and immunology, laboratory outlines in, 927
in urinary infection, 818
of bile, 1210
of colds, 1125
of excised tonsils, note upon, 988
of thyroid gland in goiter, 86
- Bacteriophage, 818
demonstration of, in old stock cultures, 1074
in sugar media, behavior of, 937
of d'Herelle, studies on, 100
toxicity of, 26
Twort d'Herelle's, prophylactic and therapeutic possibilities of, 20
- Bacterium abortus, is it pathogenic for human beings, 38
- Barbituric acid derivatives as anesthetics and methods for administration, 1071
- Basal metabolism, calcemia and glycemia in thyroid diseases with increased, 1122
determinations, mask for use in, 590
notes on, 884
- Beloved physician, book review, 1211
- Benzochol precipitation reaction in sero diagnosis of syphilis, 302
- Bile, bacteriology of, 1210
secretion, study of, from a case of biliary fistula, 1067
- Biliary antiseptic, unsuccessful experiments with mercurochrome as, 617
fistula, case of, bile secretion from, 1067
- Bilirubin, formation of, 1121
in feces and meconium of nursing infants, 190
of the blood, comparison of the quantitative methods for, 802
- Bilirubinaemia, variations of, significance of, 304
- Biologic significance of soluble specific substances of pneumococci, 303
- Birth control, 927
- Bismuth in treatment of syphilis, clinical value of, 396
- Blood, agar plate for spore forming anaerobes, 295
agglutination of, method for preventing, by glucose solutions, 369
and urine, chlorides in, rapid method for determination of, 702
nonprotein nitrogen of, Koch and McMeekin method for determination of nitrogen, 286
bilirubin of, comparison of quantitative methods for, 802
calcium, colorimetric method for the estimation of, 718
in eclampsia, 504
cells, photographic method of counting, 456
white, biologic differentiation in, 304
changes in rabbits resembling those in pernicious anemia accompanying B Welchii infections, 922
chemical changes in pertussis, 514
in pertussis, significance of, 711
chemistry, colorimetric methods, 1131
in eclampsia, 714

Blood—Cont'd

cholesterol findings in syphilis and in other skin diseases 518

coagulation time and retraction time of method for the determination of 1113

color index of chart for reading 1108

counts, normal in pigeons 437

culture technique, a simple 607

cultures, observations on with special reference to the quantity of the blood used, 145

cyanate in the 617

during pregnancy, lactic acid content of 814

for chemical examination note on the measurement of, 911

study preservation and transportation of 79

glucose curve, mathematical derivation of the, 318

in the method for estimation of 96

glycolysis and lactic acid formation on rabbit, 240

studies in 239

groups in general paralysis 410

hemochromogen crystal test for 96

hemoglobin content of standard method of recording 889

hemolyzed method of preserving 611

human, large mononuclear and the transitional leucocyte of 71

in cases of hypertension 512

in smallpox during a recent epidemic 1123

iron and hemoglobin in colorimetric method for the determination of 917

iron in 620

morphology, changes in 97

plasma determination of fibrin in 1092

platelets and corpuscles effect of pneumococcus extract on 504

volume measurement of 282

pressure 814

and urinary findings in normal pregnancies, 816

in rabbits method for estimating 106

proteins tungstic acid precipitation of 800

sedimentation, 018 712

relation of to pelvic disorders 89

test graphic presentation of the 618

smears stand for staining 91

stain a modified Schuffner's 93

staining, influence of hydrogen ion concentration on 1129

stream inoculation smallpox without eruption following 820

sugar 432 618 712

apillary, method for 906

outlet and insulin treatment of derma to e. 1121

curves in epidemic encephalitis 504

determination of critical examination or four methods commonly used for the 403

effect of injections of insulin and dextrose on 820

estimation of comparison of Folin Wu and new Benedict methods for 1125

Blood, sugar estimation—Cont'd

quantitative studies of 609

in pernicious anemia, 190

in status thymicolymphaticus 390

transfusion in pernicious anemia 299

uric acid blood uric oxidase and the true value of 819

in nephritis, 10

uric oxidase and the true value of the blood uric acid 819

urine density index, 1100

Body resistance leucocytic indices of, with report of a new index 482

Book reviews 101 196 306 407 520, 022, 8 9, 927 1021, 1131

Breast cancer of bacterial flora of 616

varying degrees of malignancy in, 98

Browder aspects of allergy 1213

Bronchitis spirochetal, successfully treated with arsenphenamine, 55

Buffer solutions treatment of acid intoxication with 467

Burns pathogenesis of death from 336

pathology of 185

C

Calcemia and glycemia in thyroid disease with increased basal metabolism 1122

Calcium in serum content of 303

content of serum of normal adults 157

in tissues feces, and milk, determination, of 399

California hay fever plants 1164

Cancer diagnosis of, use of tannin in 1020

metastasis mechanism of, 924

of the breast bacterial flora of 616

varying degrees of malignancy in 98

organisms repeated inoculations of animals with 616

Cannula metal an improvised 1011

Capillary blood sugar, method for 906

circulation influence of upon certain allergic conditions 1149

Carbohydrate metabolism new views on, 719

mediums stability of 474

Cirrhosis papillary type 220

primary of the lung 89

Cellulose as an aid in the determination of members of the colon aerogenes group of bacteria 290

Cell red blood normal and pathologic fragmentation of 925

Cerebrospinal fluid P₁₀ of colorimetric method for the determination of 396

chlorides of the 49

diagnostic value of the sugar content in 818

infections in the in the course of serum reactions 117

organic phosphorus of 84

quantitative estimation of the total protein content in 1129

sugar and chloride content of, in neurosyphilis 43

test 336

value of routine examination of 61

syphilis effect of treatment on spinal fluid in 299

- Chambers apparatus, an accessory to, for the isolation of single bacterial cells, 795
- Chemical analysis of foods, 196
- Chemicomycologic formulas, 83
- Chemistry of the therapeutic substances, advance in the, 410
- toxicologic, studies in, 773
- Chemotherapy and serum therapy of pneumococcus and streptococcus meningitis, 1013
- of gingivitis, 820
- of streptococcemia, 572
- principles and practice of, 622
- Chicken pox, leucocytic reactions in, 79
- Chloretone anesthesia of dogs, 139
- Chloride content of canned sauerkraut, 561
- Chlorides in blood or urine, rapid method for determination of, 702
- of the cerebrospinal fluid, 49
- Cholera infantum, upper respiratory infection as a cause of, 1121
- Cholin in paralytic ileus, 430
- Chorea, analytic study of, 1120
- Chorioepithelioma of ovary, 215
- Chronic pollen hay fever and asthma, differences in, in children and adults, 1197
- Circulatory respiratory functional efficiency, clinical tests for the estimation of, 823
- tomas vs circulatory depressants, 814
- Clinical interpretation of Wassermann reaction, 308
- investigations in allergy, 1159
- laboratory methods, 197
- procedure, 1131
- results with pathogen, 465
- Coagulation time and retraction time of blood, method for the determination of, 1113
- Coccidioidal granuloma, report of case, with autopsy findings, 57
- Colds, bacteriology of, 1125
- Colloidal gold solutions, as applied to the Lange reaction, the correction of, 1017
- preparation of, 810
- Color index, volume index, and saturation index, tables for calculation of, based on recently determined standards 399
- Colorimeter, hydrogen ion, description of a new, 694
- the Gradwohl, 695
- Colorimetric determination of phosphorus, 300
- method for the determination of iron and hemoglobin in the blood, 917
- for determination of P_H of cerebrospinal fluid, 396
- for the estimation of blood calcium, 718
- in blood chemistry, 1131
- Complement fixation in tuberculosis, 563
- reaction in anti pollen serum, 259
- test for syphilis, method of diluting antigens for use in, 594
- Congenital syphilis in Boston, twenty five years of, 1124
- Corpuscles, protein, conduct of, as a reflection of certain diseased processes in the human organism, 507
- Cortical sclerosis, diffuse, 644
- Corynebacterium ulcerans, a pathogenic microorganism resembling *C. Diphtheriae*, 756
- Counting blood cells, photographic method of, 456
- Creatinine and uric acid in the urine of infants, 922
- rate of, when administered to man, 510
- Crystalline proteins, urinary proteins, of nephritis, 511
- Cults and their relation to medical practice, 1033
- Culture media, 715
- Cyst, dermoid, lipoidal reaction in wall of, 213
- Cystadenoma, papillary, 218
- Cystitis, incrustated, with alkaline cystitis, etiology of, 395

D

- Decalcification, new method of, 91
- Dermatoses, blood sugar content and insulin treatment of, 1121
- D'Herelle's bacteriophage, studies on, 100
- Diabetes, compensatory hypertrophy and hyperplasia of the islands of Langerhans in the pancreas of a child born of a mother with, 100
- constitutional and hereditary traits in, 1120
- melittus, pathogenesis of, relation of abdominal and rectal infections to, 616
- pathology and treatment of, 1021
- nature of, 318
- necropsy findings in, 1122
- Diabetic gangrene treated by insulin, 472
- Diagnosis, hookworm, place of the smear in, 715
- of gonorrhea by culture, 1018
- of rabies, laboratory, eliminating a source of error in, 1130
- Diagnostic case, a useful, 1124
- Diet, influence of, on the physiologic assay of insulin, 256
- Dietetics, practical, in health and disease, 1022
- Digestion acid, a modifier, for nonprotein nitrogen determination, 387
- Dihydroxyacetone, quantitative determination of, 400
- Diphtheria, immunization against, with toxin detoxified with sodium ricinoleate, 89
- Diplococcus from measles, studies on, 97
- Disease, intraperitoneal, acute, abdominal puncture in the diagnosis of, 403
- Diseases of the heart, 822
- Dogs, chloretone anesthesia of, 139
- Dreyer's defatted antigen, unsuccessful attempts to cure or prevent tuberculosis in guinea pigs with, 305
- Drop recorders, 686

F

- Fetalsmia, 714
 blood calcium in 504
 chemistry in, 714
 Edema 711, 713
 Electrolytes and nonelectrolytes influence of, on bile solubility of pneumococci, 96
 Encephalitis, epidemic blood sugar curves in, 504
 studies on the etiology of 1123
 Endocarditis and auricular fibrillation, case report of 35
 Endometrial histolytic by culture practical value of examination for 503
 Endotheloid cell in acute leucemia 855
 Enzymes, 521
 Eosinophilia in asthma hay fever and allied conditions 1145
 Ephedrin, 624
 treatment of hay fever with 1203
 Epidemiology of pneumonia, 309
 Epidermal group of proteins treatment of asthmatic patients affected by 1179
 Epilepsy, spinal fluid in 819
 Erysipelas antistreptococci serum etiology and treatment with 403
 Erythrocyte sedimentation reaction relation of, to the ability of flocculation of the plasma and serum 712
 Erythrocytes antigenic nature of duality of, 979
 and hemoglobin in the south 679
 Etiology, and epidemiology of impetigo contagiosa neonatorum, 97
 and pathology of granuloma inguinale 508
 of peptic ulcer 921
 and treatment of pernicious anemia 921
 with erysipelas antistreptococci serum 403
 of granuloma inguinale 929
 of incrustated cystitis with alkaline cystitis 395
 of malignant neoplasms, 1013
 of tumors, 296
 Examination of cerebrospinal fluid value of routine 615
 Exsanguination transfusion in treatment of phenol poisoning 313
 Extrinsic asthma, 1187
 Exudate from nutrient agar slants 715

F

- Fat of feces determination of amount and composition of 294
 Fats and lipoids, an aldehyde mordant for 91
 Feces and meconium bilirubin in of nursing infants 190
 parasitic ova in, cedar oil as an aid in finding 1117
 Fibrillation, acute auricular, case report of 33, 38
 auricular, and endocarditis, case report of, 34
 Fibroadenoma 218
 glandular type 221
 nonglandular type, 321

- Fibroma arising from wall of blood vessel 221
 degenerative change in 222
 sarcomatous degeneration of 224
 Filter candles effect of paraffin and oil substances upon, 148
 Flocculation in tuberculosis, 193
 tests in the serology of syphilis 891
 Food poisoning bacteria, differentiation of 90
 Foods chemical analysis of, 196
 Folliculoma ovarii 216
 Folin Wu blood sugar values, correction of, 398
 and new Benedict methods, for the estimation of blood sugar comparison of, 278
 comparison of, for sugar in blood and cerebrospinal fluid, 712
 Footprints, 1217
 Formaldehyde sulphuric acid reaction of the opium alkaloids, 773
 Formulas chemicoanatomic, 83
 mycologic 82
 French azure C tissue stain modification of 511
 Frozen section methods difficulties and value of, 492
 sections new method of mounting fixed, 1014
 Fungi of blastomycosis infective and significance of a newly described ascospore state for the 501

G

- Gangrene diabetic, treated by insulin 474
 Gasometer gas analysis method simplified calculation for 884
 Gas poisoning sodium thiosulphate and calcium salts in prevention of the sequelae of illuminating 109
 Gastric movements in the pigeon with economy of animal material 1004
 secretion in nephritis 362
 Gastro duodenal ulceration, surgery of 944
 Gentian violet solutions preparation of, for intravenous injection 78
 Gingivitis chemotherapy of, 820
 Glomerulonephritis acute, experimental production of 815
 Glucose in the blood method for estimation of 96
 in the urine fallacies of the yeast method of detecting, 80
 tolerance test 389
 utilization in renal glycosuria 506
 Glycerol chloroform anesthesia 882
 notes on, 882
 Glycolysis blood on rabbit 240
 blood studies in 239
 on ox blood in vitro 240
 Glycosuria 1128
 renal glucose utilization in 506
 Goiter bacteriology of thyroid gland in 86
 exophthalmic histologic changes following administration of iodine in 821
 nonsurgical types and treatment 823

- Gonococcus* and *meningococcus*, cultivation of, 405
 in man, agglutination of, 716
 white of egg culture medium for the, 79
 vaccines with urotropin, 95
Gonorrhea, diagnosis of, by culture, 1018
 Gridwohl colorimeter, 695
 Guin stim, notes on, 405
 Granuloma, coecidoidal, report of case with autopsy findings, 37
 studies in, 1020
 inguinal, etiology and pathology of, 506
 etiology of, 926

II

- Hay fever, and asthma, 825
 differences in, in children and adults, 1197
 cosmophilism, 1145
 plants of California, 1164
 treatment of, with ephedrin, 1203
 Heart, action of indol and skitol on the, 991
 and vascular disease, bacteria in, elective localization of, 31
 diseases of, 822
 Hematoxylin eosin staining method for paraffin sections, 515
 Hemendothelocyte, 74
 Hemochromogen crystal test for blood, 96
 Hemoglobin and erythrocytes in the South, 679
 content of blood, standard method of recording, 889
 Hemolyzed blood, method of preserving, 611
 Hemophilus, blood clotting studies in, 1122
 Hemorrhages, their significance and methods of treatment, 921
 Heredity, influence of, on atopy, 1135
 Hexylresorcinol, in treatment of pyelitis of infancy and childhood, 401
 in urology, clinical application of, 401
 Hip joint, tuberculous disease of, diagnosis, treatment and end results of, 410
 Hookworm, diagnosis, place of the smear in, 715
 infections, evaluation of the methods of Stoll and Lano in, 715
 estimation of the number of, harbored, by the use of the dilution egg count method, 716
 Hospital laboratory work, integration of, 666
 Hydrogen ion colorimeter, description of a new, 694
 concentration, 522
 determinations, buffer mixture for the alkaline range of, 194
 influence of the, on blood stamug, 1129
 of capillary blood, micromethod for estimation of, 293
 Hypertension, blood in cases of, 512
 ultimate, results of essential, 814
 Hypercholesterolemia, 189
 Hypersensitiveness, studies in, 1135
 Hypo and hypercalcemia, experimental, state of serum calcium in, 514

I

- Icterus index, modification of, 813
 Immune properties, isolation of substance with, 619
 Immunity, 619
 relation of anaphylaxis to, 505
 rôle of the reticulo endothelial system in, 716
 Immunization against diphtheria with toxin detoxified with sodium ricinoleate, 89
 against scarlet fever, active, dosage of toxin for, 511
 of the pneumococcus, 504
 of rats against pneumococcus by feeding the acid killed germs and the influence of the age of the animal thereon, 566
 against scarlet fever with toxin detoxified with sodium ricinoleate, 89
 of sheep by means of anthrax bacilli attenuated with sodium chloride, 926
 Immunology, and bacteriology, laboratory outlines in, 927
 Impetigo contagiosa neonatorum, etiology and epidemiology of, 97
 Indol, and skitol, action of, on the heart, 991
 oxalic acid test for, 501
 Infantile progressive muscular atrophy, early, clinical and pathologic study of cases, 837
 Infection, urinary, bacteriophage in, 818
 Infectious, abdominal and rectal, relation of, to the pathogenesis of diabetes mellitus, 616
 of the skin and its appendages, superficial yeast, 395
 Insanity and law, 197
 Insulin, and dextrose, effect of injections, on blood sugar, 820
 diabetic gangrene treated by, 472
 hypodermic, effect of, in normal subjects, 116
 effect of, in diabetic subjects under control, 117
 in normal subjects, 116
 in underweight normal subjects, 117
 on fasting blood sugar in normal and diabetic subjects in relation to percentage normal weight, 115
 physiologic assay of, influence of diet on, 256
 Interagglutination of maternal and fetal blood in the late toxemias of pregnancy, 617
 Intestinal amebiasis from the pathologist's standpoint as related to the clinical picture, 451
 antiseptics, action of certain alleged, 397
 putrefaction and indican output, effect of bacillus acidophilus on, 226
 Intradermal salt solution test in normal and toxemic pregnancies, 713
 Intraperitoneal disease, acute, abdominal puncture in diagnosis of, 403
 Intrinsic asthma, 1191
 Iodine, histologic changes following administration of, in exophthalmic goiter, 821

- Iron and aluminum pharmacology of in relation to therapeutic use 190
in cooking utensils 191
pharmacology of in relation to therapeutic uses 730
and hemoglobin in the blood colorimetric method for the determination of 917
hematoxylin method a quick 40
in biologic materials quantitative method for the determination of 401
in blood 620
tissues and urine determination of 40
Isopropyl alcohol experiments of human beings 336
in preparation of Wassermann antigen 52
physiologic properties of 36

J

- Jahnel's method for staining peripheral nerve tissue 9
Jaundice method of estimating the amount of by means of the bilirubin 505

K

- Kahn flocculation test comparison between the Kolmer Wassermann test and the Ruckiger Wassermann test 269
precipitation test device for the detection of antigen in 1096
rapid mixing flasks for the preparation of the standard antigen dilution used in the 182
Kaiserling method for preserving gram specimens modification of 1
Keratitis bacillus pyoverdineus experimental 442
Kidney diseases 203
Kidneys physiologic and anatomic changes following temporary ischemia 349
Klotzman's reaction based on tubulin and biologic tests 1193
Koch and McMeekin method for determination of nitrogen with special reference to nonprotein nitrogen of blood and urine 286
bacillus staining methods for 114
Koch's bacillus bacteriologic examination of the urine for 186
Kolmer quantitative test comparison of with the routine Wassermann test a simplification not a modification of 1109
Kottmann reaction diagnostic value of in thyroid dysfunction 713
Kramer Gittleman modification of the method of Fohn and Wu study of the accuracy of 432
Kromograph long paper new type of motor driven 170

L

- Lacetic acid content chemical changes in the spinal fluid in meningitis with special reference to 81a

- Laboratory examinations—not laboratory tests 1133
work hospital integration of 666
Lactobacillus acidophilus 201
cultural characteristics of 1053
Lange reaction colloidal gold solutions as applied to correction of 1017
Lard chloride intravenous administration of 559
Leptospira pathology of 112
Leukemia acute endotheloid cell in 800
in the central nervous system 1013
Lymphocyte counts differential in the counting chamber combined diluting and staining fluid for 148
of human blood transitional 71
Lymphocyte large mononuclear 73
Lymphatic indices of body resistance with report of a new index 487
infections in chicken pox 79
in measles 1
in mumps 1
in scarlet fever 1
in smallpox 9
Lymphatic reaction in wall of terminal cyst 13
Lymphatic test 185 18 3
tumors of the four unusual malignant 100
Lymphatic lymphatic passage in 512
Lymphatic lymphatic studies in 993
Lymphatic lymphatic culture for direct isolation of the tubercle bacillus from sputum 15
Lymphatic milk Wassermann and flocculation reaction in 113
Lymphatic test a specificity of the 513
Lymphatic lymphatic of the 59
Lymphatic lymphatic with formation of abscess in guinea pigs due to infection with hemolytic streptococcus 111
Lymphatic lymphatic 3
Lymphatic lymphatic 1119
Lymphatic lymphatic duration of disease and the effect of roentgen rays and radium irradiation in surgery 181

M

- Mackenzie the Beloved Physician 1211
Magnesium salts influence of on antibody and complement titrations 13
Malaria in the treatment of general paralysis 955
Manganese in urine its detection and determination 709
Milk for use in basal metabolism determination 790
Mikrokin test in syphilis study of 946
Moral's leptococcus in 17 98
leucocyte reactions in 79
prevention of by immune goat serum 9
prophylaxis use of blood from convalescents in a school epidemic 111
Mikrokin differential use of potassium tellurite in 194
special liquid simplified method for the preparation of by filtration 1009

- Pollen antigens, comparative study of, as determined by skin reaction, 1139
 content of the air, factors which determine, 1151
 grains, structural features in identifying, 1164
 oral administration of, 1156
- Polveythemia vera, ease of, treated with phenylhydrazin hydrochloride, 597
- Potassium and sodium tetrabismuth tartrates, preparation of, 962
 pyrogallate solution, preparation of, for metabolic rate determinations, 287
 tellurite, use of, in differential media, 194
- Pregnancies, normal and toxic, intradermal salt solution test in, 713
 blood pressure and urinary findings in, 816
- Pregnancy, early diagnosis of, by precision, 615
 interagglutination of maternal and fetal blood in the late toxemia of, 819
 lactic acid content of the blood during, 814
 toxemia of, acidosis in, 816
 toxemias of, interagglutination of maternal and fetal blood in the, 617
- Preparation of high class nutrient media for the cultivation of germs which are very difficult to grow, 84
- Prescription notes, 307
- Preventive medicine, private practitioner as pioneer in, 308
- Principles of diagnosis and treatment in heart affections, 1211
- Protein, corpuscles, conduct of, as a reflection of certain diseased processes in the human organism, 507
 of the epidermal group, treatment of asthmatic patients affected by, 1179
- Proteins, of blood plasma, effect of venous stasis on the, and on the rate of sedimentation of the red blood corpuscles, 735
 urinary, crystalline, of nephritis, 511
- Proteus, bacteriologic investigation of in material containing, 510
- Psoriasis, isolation of monilia from the skin scales, mouth, and sputum in, 97
- Puerperal fever, streptococcus hemolyticus toxin and antitoxin, 305
- Pulmonary arteritis, 409
 artery, sclerosis of, 411
- Pneumonia of infancy and childhood, hexylresorcinol in treatment of, 401
- Pigmentation or the doctor of the future, 200
- Q
- Quantitative, blood sugar estimation, studies of, 609
 determination of dihydroxyacetone, 400
 methods for the bilirubin of the blood, comparison of, 802
 for the determination of iron in biologic materials, 401
- R
- Rabies, eliminating a source of error in the laboratory diagnosis of, 1130
- Radium, therapeutic uses in general practice, 306
- Rapid mixing flasks for the preparation of the standard antigen dilution used in the Kahn precipitation test, 182
- Rat bite fever, cultivation of the spirillum of, 97
- Reaction, anticomplementary, of blood serum to the Kolmer complement fixation test controlled by the reaction to the Kahn precipitation test, 265
 benzochole precipitation, in the sero diagnosis of syphilis, 302
 complement fixation, in antipollen serum, 259
 formaldehyde sulphuric acid, opium alkaloids, 773
 Kottmann, diagnostic value of, in thyroid dysfunctions, 713
 lipoidal, in wall of dermoid cyst, 213
 skin, in polymyelitis, 818
 starch iodine, value of, as a test of pancreatic function, 297
 Wassermann, chemical interpretation of, 308
 in rabbit syphilis, 924
 Van Den Bergh, for serum bilirubin with notes on interpretation and technique, 529
- Red blood cells, normal and pathologic fragmentation of, 925
- Renal glycosuria, glucose utilization in, 506
 insufficiency, relationship between memory and, 512
- Reports of St Andrew's Institute for Clinical Research, 1211
- Respiratory infection, upper as a cause of cholera infantum, 1121
 tract, monilia from the, 968
- Rheumatic fever, an illytic study of, 1120
- Rhinitis, allergy, 1162
- Ringworm fungi, cultural study of, 86
- Romazowsky Giemsa stain, buffered water for, 510
- Rubber mask for determination of oxygen consumption of the dog, 378
- Russell double sugar tube, mechanism of, 94
- Russian thistle pollen, 1142
- S
- Saliva, urea and ammonia nitrogen of, quantitative method for the determination of, 1015
- Salivary glands in teratoma, 215
- Salmonella muenchster, infection of man, probably due to, 141
- Sarsaparilla, emmed, chloride content of, 161
- Sarcoma, polymorphous cell, 224
 spindle cell, with little stroma, 224
- Scarlet fever, 512
 antitoxin, preparation and chemical application of, 821
 control of, in institutions, 816
 dosage of toxin for active immunization against, 511

- Scarlet fever—Cont'd
 immunization is most with toxin de-
 toxified with sodium ricinoleate 89
 leucocytic reactions in 79
 streptococcus scarlatinae in role of the
 washings of 274
- Schuffner's blood stain, modified 93
- Sclerosis cortical diffuse 644
 of the pulmonary artery 411
- Sedimentation blood 618 714
 of the red blood corpuscles effect of
 venous stasis on the proteins of
 blood plasma and on the rate of
 735
 rates, 739
 rate of erythrocytes the corpuscle volume
 and the icterus index 313
 test blood graphic presentation of the
 618
 in obstetrics and gynecology 80
- Serodiagnostics of syphilis benzochol precip-
 itation reaction in 302
- Serology of syphilis flocculation tests in
 891
- Serum, antipneumococcus specific precipi-
 tates obtained from and antibody
 solution by the soluble specific sub-
 stances of pneumococci, 303
- antipollen complement fixation reaction
 249
 applied in gynecology, Lank and Huer's
 photochemical test of 190
 bilirubin Van Den Bergh reaction for
 529
 calcium in, content of 303
 erysipelas antistreptococci etiology and
 treatment with 403
 method of collecting small amounts of
 184
 of normal adults calcium content of 17
 Sheep cells preservation of for use in com-
 plement fixation tests 255
- Skin reaction in poliomyelitis 819 114
 measles, 98
 of the toxins from hemolytic strepto-
 cocci from puerperal and scarlet
 fever comparison of 404
 pollen antigens determined by 1139
 with filtrate of Koch strain of bacillus
 tuberculosis 513
 tests in chronic nephritis 1
- Smallpox, leucocytic reactions in 79
 the blood in during a recent epidemic
 1123
 with eruption following blood stream in
 occlusion 820
- Smears stained viability of plague bacilli
 in 19
- Sodium thiosulphate and calcium salts in
 prevention of sequelae of inflammation
 and poisoning 109
- Solutions gentian violet preparation of for
 intravenous injection, 78
- Specimens gross modification of the
 Krausling method for 517
- Sphygmomanometer recording, an adjust-
 able sphygmoscope for the 173
- Spinal fluid and blood chlorides in 49
 in epilepsy 819
 tryptophane reactions in 1048
- Spirocheta dentium cultivation of pure
 cultures of 187
 pillula cultivation of pure cultures of
 187
 immunologic strains of 94
 purification of cultures of, by chemical
 methods 1017
 recurrent cultivation of pure cultures
 of 187
 bronchitis, successfully treated with
 sphenimine, 55
- Sputum examination in so called closed
 tuberculosis, 89
- Stain for nerve tissue rapid, 516
 French azure C tissue, modification of
 511
 Romanovsky Ciernia buffered water for
 510
- Stained smears, viability of plague bacilli
 in 195
- Staining acid fast and gram, error in due
 to petrolatum 97
 fish a new 716
 of bacterial flagella, method for the 404
 of eutimbria in sections technique for se-
 lective 398
 method for paraffin sections rapid hemi-
 atoxyluousin 515
 methods for the Koch bacillus 514
 methyl Weigert fast technique for 406
 technique 716
- Status thymicolymphaticus blood sugar in,
 395
- Still and Jase methods evaluation of in
 light hookworm infections 715
- Streptococci hemolytic, separation of a
 soluble specific substance from, 94
- Streptococcus chemotherapy of, 572
- Streptococcus scarlatinae in scarlet fever
 role of the washings of 274
- Stroma ovarii 222
- Structural features of pollen grains rôle in
 identifying the most important dry
 fever plants in California 1164
- Sugar and chloride content of cerebro-
 spinal fluid in neurosyphilis 43
 content in the cerebrospinal fluid diag-
 nostic value of 858
 in blood, 432
 and cerebrospinal fluid comparison of
 the Folin Wu and the new Benedict
 method for, 712
 determination of critical examination
 of four methods commonly used for
 403
 in normal urine determination of 579
 media bacteriophage in behavior of, 934
 tolerance tests, further observations of
 in pregnancy 615
 tubercle bacilli double mechanism of, 94
- Sulphamoxydiazine, 95
- Surgical conditions acute differential count
 in 89
- Syphilis and other kind diseases, blood
 cholesterol findings in 519
 cerebrospinal effect of treatment on spinal
 fluid in 299
 complement fixation test for method of
 diluting antigens for use in 594

Syphilis—Cont'd

- congenital, in Boston twenty five years of, 1124
- Meinecke microreaction in, 58
- micro Kahn test in, 946
- microscopic slide precipitation test for, 87, 477
- of the central nervous system, 861
- rabbit, Wassermann reaction in, 924
- serology of, flocculation tests in, 891
- serodiagnosis of, benzochol precipitation reaction in, 302
- serum diagnosis of, error of biasing, on the Kahn reaction alone, 296, 505
- the Vernes flocculation test for, 1127
- treatment of, chemical value of bismuth in, 396
- Wassermann test for, studies in, 303
- Syphilitic alopecia, its relation to neurosyphilis, 295, 519
- antigen, methods for making a stable emulsified, 908
- patients, study of testes from, 1124
- Syphilology, modern clinical, 520

T

- Tannin, use of, in diagnosis of cancer, 1020
- Tartrates, potassium and sodium tetrabis muth, preparation of, 962
- Techne for selective staining of eumetachrom sections, 398
- Terratom, choroid plexus in, 212
- pacchionian bodies in, 212
- retinal pigment in, 213
- salivary glands in, 215
- squamous cell epithelioma arising in, 217
- thyroid tissue in, 214
- Test, blood sedimentation, graphic presentation of, 618
- cerebrospinal fluid, 396
- cutaneous, for a hydrophobic condition, 711
- for syphilis, microscopic slide precipitation, 477
- glucose tolerance, 380
- hemochromogen crystal, for blood, 96
- intracutaneous salt solution, its use as a test of the efficiency of the circulation in the extremities, 193
- intra dermal salt solution, in normal and toxemic pregnancies, 713
- Kahn precipitation, rapid mixing flasks for the preparation of the standard antigen dilution used in the, 182
- Kolmer, a simplification, not a modification of, 1109
- quantitative, comparison of, with the routine Wassermann, 296
- Lauge and Huer's photochemical, of serum applied in gynecology, 190
- liver functional, 523
- luerin, nonspecificity of, 513
- micro Kahn, in syphilis, study of, 946
- microscopic slide precipitation, for syphilis, 87
- of pancreatic function, value of a starch iodine reaction as a, 297
- oxalic acid, for indol, 501

Test—Cont'd

- quantitative, of digestive pancreatic activity easily applied clinically, 190
- sedimentation, in obstetrics and gynecology, 99
- simple, of diagnostic value in general paresis, 99
- tuberculin, new method for, 817
- Wassermann, for syphilis, studies in, 303
- Testes, study of, from syphilitic patients, 1124
- Tests, clinical, for the estimation of circulatory respiratory functional efficiency, 828
- complement fixation, preservation of sheep cells for use in, 255
- flocculation, in the serology of syphilis, 891
- liver functional, 188, 518
- ring and flocculation, in tuberculosis, 1023
- skin, in chronic nephritis, 1
- tuberculin, 617
- Wassermann and Kahn, comparison of, 1000, run in parallel, 1087
- Tetraethyl lead and inorganic lead salts, toxicity of, 554
- oral administration of, 558
- Therapeutics, materia medica and pharmacy, 1132
- Thermoelectric apparatus, portable, for the determination of surface and tissue temperatures, 913
- Throat infections with streptococcus scarlatinae without a rash, 94
- Thromboangitis obliterans, case report of, 37
- Thyroid disease, 713
- dysfunctions, diagnostic value of the Kottmann reaction in, 713
- gland, 1021
- bacteriology of, in goiter, 86
- metastasis to, from endothelial myeloma of bone, 878
- tissue in teratoma, 214
- therapy and thyroid tolerance in chronic nephritis, 815
- Timothy pollen, 1143
- Tissue, fresh, new method of examining, 1035
- rapid paraffin embedding of, 515
- Tissues, feces and milk, determination of calcium in, 399
- Tonsils, excised, bacteriology of, 988
- Toxemia of pregnancy, acidosis in, 816
- Toxemia of pregnancy, late, interagglutination of maternal and fetal blood in, 617
- Toxicity of tetraethyl lead and inorganic lead salts, 554
- Toxicologic chemistry, studies in, 773
- Toxicology, memoranda of, 102
- Toxin antitoxin, hypersensitivity to its protein content, 88
- precipitate of Ramon, antigenic value of, 503
- Treatment of acid intoxication with buffer solutions, 467
- of asthmatic patients affected by protein of the epidermal group, 1179

Treatment—Cont d
 of general paralysis malaria in the 120
 of hay fever with ephedrin 1203
 of pernicious anemia treatment of 72
 of phenol poisoning exhumation trans-
 fusion in 311
 of pneumonia 931
 of pyelitis of infancy and childhood her-
 yrisoremol in 401
 of syphilis bismuth in 310
Treponema pallidum cutaneous tests with
 suspensions of 314
Treponematosis as seen in the rural popu-
 lation of Haiti 670
 Tryptophane reactions in the spinal fluid
 1048
 Tubercle bacilli method for obtaining single
 colonies of 9
bacillus carbon dioxide in the growth of
 90
 from sputum Lowenstein procedure
 for direct isolation of 98
 Tubercles culture of in the diagnosis of
 tuberculosis 1015
 Tuberculin active principles of prepared
 from nonprotein substances 1014
 human and bovine intracutaneous test
 with 617
 old, preparation of reduced antigens from
 505
 specific element of chemical study of
 505
 standardization of 169
 test new method for 81
 tests, 617
 Tuberculous disease of the lung joint di-
 agnosis treatment and end result
 of, 410
 meningitis 863
 Tuberculosis 717
 closed sputum examination in 89
 complement fixation in 563
 culture of tubercles in the diagnosis of
 1018
 flocculation in 193
 in guinea pigs unsuccessful attempt to
 cure or prevent with Brewer's d
 fatty antigen 300
 ring and flocculation tests in 10
 vital staining in experimental 510
 Tumor Krunkenberg 216
 Tumors etiology of 296
 of the ovary analysis of 211 500
 of the liver four unusual malignant 100
 malignant of the adrenal in children 10
 Tungstic acid precipitation of blood pro-
 teins note on 800
 Twort D'Herelle's bacteriophage prophylac-
 tic and therapeutic possibilities of
 20
 Typhoid paratyphoid carriers experimental
 617

U

Ultraviolet light, some recent works on 1212
 Urine albumin and sugar in apparatus for
 rapid quantitative routine determi-
 nation of 1205
 bacteriologic examination of for Koch's
 bacillus 18

Urine—Cont d
 acid and alkaline comparison of values
 on 586
 glucose in the, fallacies of the yeast
 method of detecting 80
 manganese in detection and determination
 of 509
 method of determination of iron in 620
 normal comparative values on, 580
 determination of sugar in, 579
 of infants uric acid and creatinine in
 922
 preservatives 1098
 reducing substances in 15
 United States public health service 618
 Urea and ammonia nitrogen of saliva quan-
 titative method for the determina-
 tion of 1011
 Uremia 611
 Uric acid and blood sugar determination
 of new titrimetric principle and
 its application to 618
 and creatinine in the urine of infants
 92
 blood in nephritis 10
 urinary infection bacteriophage in 818
 proteins crystalline proteins of nephritis
 111
 sediment number of formed elements in
 924
 sediment 926
 Uric glycosuria original in clinical applica-
 tion of 401
 Urinary gonococcus vaccines with 95

V

Vaccine gonococcus with urotropin 90
 Vaginal flora during childhood and puberty
 821
 Van Den Bergh reaction for serum bilirubin
 with note on interpretation and
 technique 329
 Venous stasis effect of on the proteins of
 blood plasma and on the rate of
 sedimentation of the red blood cor-
 puscles 73
 Vernes flocculation test for syphilis 111
 Vibrios from feces method of isolating and
 cultivating 302
 Volume measurement of blood platelets 122

W

Wassermann and flocculation reaction in
 lactic milk 1123
 and Kahn tests comparison of 10 000 run
 in parallel 1087
 antigen origin and nature of the 92
 antigens isopropyl alcohol in preparation
 of 52
 reaction cells and globulin content re-
 lationship of the and the colloidal
 precipitation reaction in spinal fluid
 in syphilitics 762
 clinical interpretation of 308
 in rabbit syphilis 924
 reactivation of 1126

Wissermann—Cont d

- slightly positive, menace of the, 627
- technic, a comparison of antigens in, 865
 - associated considerations of, 865
 - three phases of, 865
- test for syphilis, studies in, 303
- tests, 156

Weigert Paul technic for staining myelin, 406

White cell diluting fluid, ovale and a good, 614

- Willis floatation method, accuracy of, in diagnosis of light hookworm disease, 715

X

- X-ray and radium, introduction to the study of, 407

Y

- Yeast method of detecting glucose in urine, fallacies of the, 80



BACTO-LOEFFLER'S BLOOD SERUM

The uncertainty of source of supply is eliminated in the laboratory when Bacto-Loeffler's Blood Serum is used. A supply can be kept on hand ready for any emergency. Within an hour any quantity of medium may be prepared and sterilized.

Solution is made by dissolving in the proportion of 80 grams of Bacto-Loeffler's Blood Serum to 1000 c c of water. A small amount of Hemoglobin is retained in the medium. Slants are slightly buff colored and support luxuriant growth of characteristic types of *C. diphtheriae*.

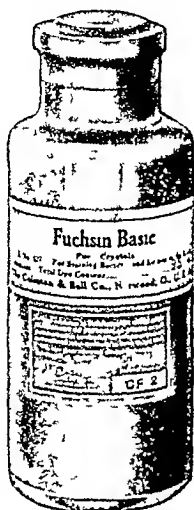
Coagulation and sterilization in the autoclave are readily accomplished by following directions given on the label. Other methods may be used with equal success.

Specify DIFCO

THE TRADE NAME OF THE PIONEERS

In the research and development of Bacto-Peptone and Dehydrated Culture Media

DIGESTIVE FERMENTS COMPANY
DETROIT, MICHIGAN, U S A



COLEMAN & BELL BIOLOGICAL STAINS

Are recognized throughout the United States and foreign countries as being *dependable*. This is due to the high standards of quality enforced in the manufacture, purification and testing of these products.

A number of the common stains are now available with the certificate of the Commission on Standardization of Biological Stains.

A complete list of Coleman & Bell Biological Stains, Chemical Indicators and Solutions is carried in stock—available for immediate shipment.

Schwarz & Company

MANUFACTURERS AND DEALERS

LABORATORY SUPPLIES, SCIENTIFIC INSTRUMENTS AND CHEMICALS

556-558 West Jackson Boulevard

Chicago, U. S. A.

Standardized Laboratory Reagents Dehydrated Culture Media

for the laboratory worker.

Our Reagents are prepared under expert direction from the purest chemicals. They are marketed in neat and usable containers.

Our Dehydrated Culture Media are made from BEEF—Not Beef Extract—with Wittie's Peptone.

No filtration is used in the process—thereby insuring the presence of "growth-stimulating" substances.

Write for complete price list and literature

GRADWOHL LABORATORIES

3514 Lucas Avenue

St. Louis, Missouri

R B H GRADWOHL, M D, Director

THE KIMBLE VENULE



Recognized by the medical world

RECOGNITION of the Kimble Venule was due to its simple expeditious method of operation and because its scientific design sterile manufacture and sanitary operation banished the danger of infection from both the patient and the operator. Value was also apparent in that it is a sterile syringe for collecting the blood specimen and a sterile container for transporting the specimen thereby eliminating the risk of contamination and erroneous results.

In case any difficulty is experienced in procuring the New Kimble Venule write us giving your dealer's name.

Kimble Venules are made in the plain evacuated type and in three special types for use in blood chemistry as follows:

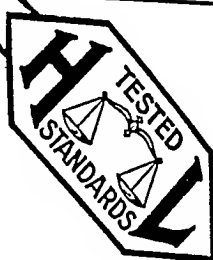
- (1) With Oxalat to prevent coagulation of the blood.
- (2) With Fluoride to prevent coagulation and to stop ferment action.
- (3) With Bile for long preservation of blood cultures for the typhoid paratyphoid group of organisms.


KIMBLE BRAND
The Visible Guarantee of Invisibly Quality
 KIMBLE GLASS COMPANY VINELAND N. J. New York Chicago

A New Type Blood Collector

VITAMINE-B

Successfully used in
PERNICIOUS ANEMIA—
PELLAGRA—ANOREXIA



*Also, in certain nutritional disturbances,
with cutaneous manifestations (see below)*

Recent reports with feeding liver and Brewers' Yeast-Harris—both rich in Vitamine-B—have shown marked benefit in cases of Pernicious Anemia

Recent articles in A M A, and other medical journals, indicate clearly the value of a diet rich in Vitamine-B, in the dietary treatment of Pernicious Anemia

The U S Public Health Service has recently announced the improvement and cures of 26 cases of pellagra in the Georgia State Sanitarium, with the addition of Brewers' Yeast-Harris to the diet

Dr Geo R Cowgill, Yale Univ, has shown *improvement in appetite*, when small amounts of Yeast Vitamine Harris are fed

Yeast Vitamine Harris Tablets are *indicated in convalescence* or typical anorexia

Lactation is stimulated and milk secretion increased by feeding liberal amounts of Yeast Vitamine Harris Tablets, according to report Dr Barnett Sure, Univ of Ark

H J Gerstenberger, Lakeside Hospital, Cleveland, Ohio, reported a series of cases of *Herpetio Stomatitis* and *Herpes Labialis*, cured with addition of Yeast Vitamine Harris Tablets to the regular diet

Goldberger and Tanner, U S P H Service, reported *cures of black tongue in dogs*, when fed Brewers' Yeast Harris (medicinal)

The Connecticut Experiment Station and U S P H Service have shown the *superiority of brewers' yeast over bakers' yeast*, as a source of Vitamine-B and as a cure for *specific disease*

Sample bottle of yeast or Yeast Vitamine Tablets To physicians only

\$1 00 each

THE HARRIS LABORATORIES

Tuckahoe, New York

Ethyl Chloride

U. S. P. 9—Imported

Convenience!

THE convenience of obtaining Ethyl Chloride in such handy, automatic lever tubes, as pictured to the right, needs no exposition. The two escape valves permit of either a dropping or squirting application. For prices write to



15-gram Tubes
30-gram Tubes
60-gram Tubes
100-gram Tubes

Pfaltz & Bauer, Inc.
300 PEARL STREET NEW YORK

Chicago

Cleveland

Boston

Los Angeles

San Francisco

LaMotte Roulette Comparator



Patent Applied For

This set represents the highest development of equipment for the determination of hydrogen ion concentration. It covers an extended pH range and

combines the greatest possible convenience, speed and accuracy. Readily portable and can be used at night as well as during the day.



Write for a copy of our interesting brochure, *The A B C of Hydrogen Ion Control*. It is free on request.

LaMotte Chemical Products Co.
420 Light St., Baltimore, Md., U. S. A.

WHEN THE "CALCIUM INDEX" IS LOW

and is the cause—direct or indirect—of various forms of ulcer, slow healing sores, or even tuberculosis, an efficient corrective is found in

Para-Spleen Co.
(Harrower)

1 sanitablet q i d

NOTE In stubborn cases supplement the oral treatment with Sol Para Spleen Co (Harrower)

The Harrower Laboratory, Inc
Glendale California

CONTENTS FOR SEPTEMBER, 1927

Clinical and Experimental

Studies in Hypersensitiveness XXIX On the Influence of Heredity in Atopy By Arthur F Coca, New York City	1135
A Comparative Study of Pollen Antigens as Determined by the Skin Reaction By Robert F E Stier and Guy L Hollister, Spokane, Washington--	1139
Eosinophilia in Asthma, Hay Fever, and Allied Conditions By Grafton Tyler Brown B S, M D Washington D C	1145
The Influence of the Capillary Circulation upon Certain Allergic Conditions By Edward Scott O Keefe, M D, Boston Mass	1149
Factors which Determine the Pollen Content of the Air By Ray M Blair M A, M D, Oklahoma City, Okla	1151
The Oral Administration of Pollen By J H Black M D Dallas Texas --	1156
Clinical Investigations in Allergy A Review of 189 Patients of Asthma and Hay Fever Clinic By Leon Unger M D Chicago-----	1159

(Continued on pages 9 and 14)

The Klett Multi-Pipette

devised by Dr J Short, New York City

Developed especially for the Kingsbury Clark Albumen test and the Benedict Sugar or Sumner Sugar test

Its advantages are as follows —

Draws and dispenses simultaneously in 16 pipettes any amount of liquid from $\frac{1}{2}$ cc to 5 cc in the smaller and from 1 cc to 20 cc in the larger type

The accuracy is fully as great as could be obtained by single pipetting

Very simple to operate eliminates mistakes and breaking of glassware

The saving of time is enormous

The Improved Clark Lamp

The new improved and low priced Clark Lamp Model FK especially designed to provide artificial daylight illumination for use with these methods, has the advantage, that only one lamp is used instead of a series of lamps as used in the original model

It is suitable for any other colorimetric work especially hydrogen ion determinations or whenever the block method is used for color comparison Ready for delivery October first

The Klett Bakelite Test Tube Rack

Heretofore the racks for boiling a larger number of test tubes were made of copper After using them for some time the seams became loose, the metal corroded and the warping made them unsuitable for the above mentioned tests

The new test tube racks are made of Bakelite which does not warp shrink, dent, or corrode keeps its good appearance and is practically indestructible

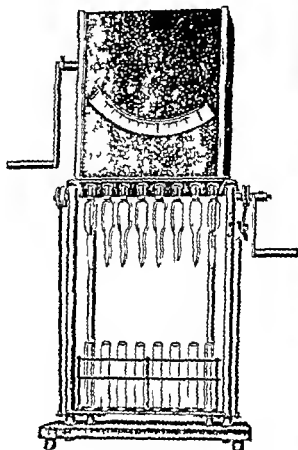
Made in three sizes one holding 16 centrifuge tubes the other two holding the Benedict sugar tubes and the 16 albumen test tubes All fit the Multi Pipette

Special sizes made to order at request

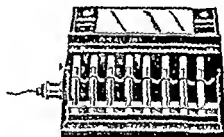
Ask for literature

KLETT MANUFACTURING COMPANY

202 East 46th Street — New York, N Y



The Klett Multi Pipette



The Improved Clark Lamp

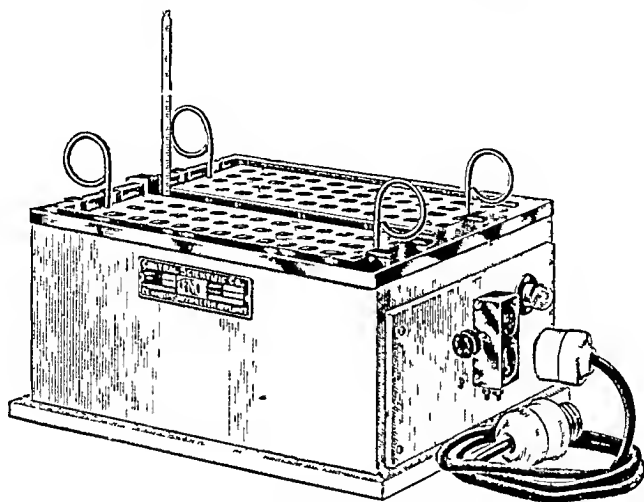
For Accurate Wassermann Work

The Temperature of Inactivation Must be Held Constant in Every Tube Between 55° and 56° C

The DeKhotinsky Electrically Heated and Regulated

Wassermann Bath

Has Been Carefully Tested and Found to be One of the Few Which Meet This Requirement



No 14336 B

The DeKhotinsky Electrically Heated and Regulated Wassermann Bath was designed to provide an easily operated, dependable bath especially suitable for routine sero diagnostic work. It may be used either as an inactivating bath at 56° C or as an incubating bath at 37.5° C, the change being made by a simple operation, which will not injure the thermoregulator, as has been the difficulty with older types of electric Wassermann Baths.

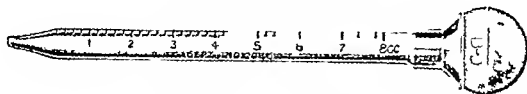
The thermoregulator is easily set for any temperature desired by turning the knurled knob on the side. When the thermometer reaches the temperature desired, adjust the thermoregulator until the light just goes out. The bath will then maintain this temperature until it is reset for a different temperature. Two trays are provided with places in each for 48 tubes up to 1/2 inch in diameter. The holes are so marked that the location of any tube may be indicated by a number and a letter. Size overall, 11 x 11 x 5 1/2 inches. Furnished for 110 volts A C or D C.

14336B Wassermann Bath, DeKhotinsky Electrically Heated and Regulated, complete with two trays, thermometer, connecting cord and attachment plug, but without glass tubes ----- \$65.00

CENTRAL SCIENTIFIC COMPANY
LABORATORY SUPPLIES
 Apparatus TRADE MARK **CSC** Chemicals
 460 E Ohio St Chicago U.S.A.

B-D PRODUCTS

Made For the Profession



This ASEPTO SYRINGE is very useful in Laboratory work for measuring and also for removing supernatant liquid

ASEPTO SYRINGES are made in forty styles and sizes. They have sensitive one hand control, fluid does not flow back into bulb and they are easily cleaned and sterilized

Send me illustrated pamphlet on Asepto Syringes

Name _____

Address _____

PD

Becton, Dickinson & Co, Rutherford, N J

Makers of Luer Syringes Yale Needles B D Thermometers Asepto Syringes and B D Manometers



- ❑ BASICALLY a Quality Instrument
- ❑ Sensitive Flexible Perish not
- ❑ Chrome Plated to resist rust
- ❑ Guaranteed to resist rust in ordinary careful use
- ❑ Inexpensive

Chromium is that element which is fused with steel in all the so called 'Rustless' or 'Stainless' varieties which give it its rust resisting power

By our new process a pure Chromium deposit or plating is achieved in place of the small percentage of Chromium used in the older method

Scientific tests have demonstrated that Chrome plating has at least five times the life and wear of nickel. It is replacing nickel plating in all the big industries such as Automobile Plumbing Tableware etc etc

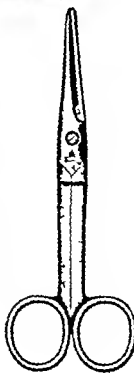
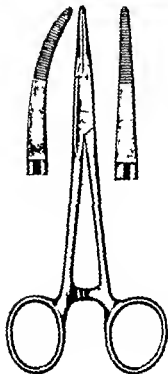
Sold through dealers only

S DONIGER & CO, Inc

23 East 21st Street

New York
Sole Agents for
Surgical Instruments

New York City



GASTRON

A Complete Gastric Gland Extract

A clinical resource against disorders of gastric function, acute, and under strain and stress of exhausting disease Gastron contains the enzymes, co-ferments, associated organic and inorganic constituents of the entire gastric mucosa, is of standardized proteolytic energy, grateful, agreeable to the stomach Prescribed simply by the name GASTRON

Fairchild Bros. & Foster
NEW YORK



POLLEN EXTRACTS

for the Diagnosis and Treatment of HAY-FEVER

STANDARDIZED TREATMENT SETS for the physician who prefers to use them

INDIVIDUAL TREATMENT SETS which enable the doctor to adjust the treatment to the particular needs of the individual patient

CONCENTRATED POLLEN EXTRACTS for the specialist who prefers to make his own dilutions

TEST SETS for determining the pollens to which hay-fever patients are susceptible

Limited quantities of all important hay-fever pollens are available in the dry form for research workers

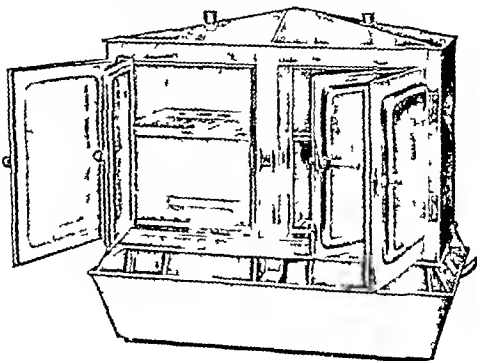
SWAN-MYERS COMPANY, INDIANAPOLIS, INDIANA
Pharmaceutical and Biological Laboratories

Mention this Journal when writing to Advertisers

The Standard for 30 Years

The principle of Arnold Sterilizers has stood the test of thirty years use. In its improved Boston Board of Health model it is the standard for laboratory use. It is made either with single or double doors or in the round side door pattern.

New Castle incubators, baths and sterilizers are also available. Send us your requirements and ask for specifications.



Arnold Sterilizers

(Steam at 100° C.)

No 638

15¼x12¼x12¼"

No 639

19½x16½x14½"

No 638—Double Form

15¼x12¼x12¼"

CASTLE



Every laboratory needs a good
sterilizer

Sterilizers Phys.

Depts. of Hospitals

WILMOT CASTLE COMPANY 1213 E. 10th Avenue, Rochester, N. Y.

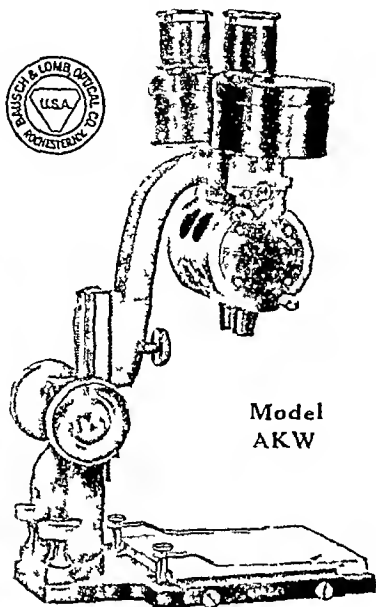
CONTENTS FOR SEPTEMBER 1927

(Continued from page 4)

- | | |
|---|------|
| The Role of the Structural Features of Pollen Grains in Identifying the Most Important Hay Fever Plant of Chicago By George Pines M.D. and H. E. McMillan A.B. M.A. Los Angeles | 1104 |
| Treatment of Asthmatic Patients Affected by Protein of the Epidermal Group By Zella White Stewart M.D. Iowa City, Iowa | 1113 |
| Studies in Asthma I. A Clinical Survey of 103 Patients with Asthma Followed for Two Years By Francis M. Blackmann M.D. Boston | 1123 |
| Essential Differences in Chronic Pollen Hay Fever and Asthma in Children and in Adults By I. S. Kahn M.D. San Antonio, Texas | 1129 |
| Further Observations on the Treatment of Hay Fever with Ephedrin By Fred W. Gaarde M.D. and Charles R. Maytum M.D. Rochester, Minn. | 1135 |

(Continued on page 14)

KW Microscope and the Drum Nosepiece



The combination of these two Bausch & Lomb products has resulted in an instrument of exceptional merit. The KW Binocular Microscope is notable for its versatility—the binocular body is interchangeable in relation to three stands designed to facilitate different types of work—its large field, high eye point, long working distance and wide range of magnification. It is recommended for dissection work and culture colony study.

The efficient Drum Nosepiece completes the instrument. Rotation of the drum in a vertical plane brings a choice of three sets of paired objectives into parfocal position. The advantage is apparent.

Send for our Wide Field Microscope folder

BAUSCH & LOMB OPTICAL COMPANY
617 St. Paul Street Rochester, N. Y.

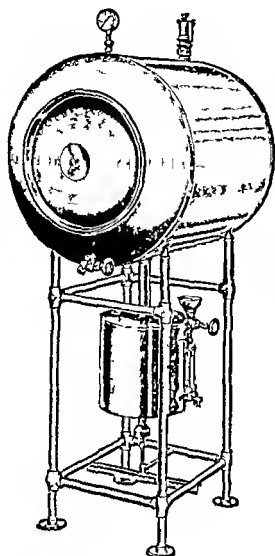
ACIDOPHYLUS TABLETS DEAN

Contain only *Bacillus Acidophylus* and pure milk sugar. To make possible intensive treatment we furnish 100 tablets in each bottle.

Price postpaid \$1.00

Dean Laboratories, Inc.

48th & Walton Ave
Philadelphia



HORIZONTAL PRESSURE STERILIZERS

GAS
ELECTRIC
STEAM HEATED

Horizontal Pressure Sterilizers, BD Type are being used in increasing numbers in bacteriological and similar laboratories, owing to ease of operation and efficient performance. Sterilizers are well constructed and built for service. Door and seat are well ground so that no gaskets need be employed for steam tight joint.

15792 Horizontal Sterilizers, with generator for gas heating

Diameter inches	17	21	23	26	28
Length inches	20	24	28	28	30
Each	\$230 00	240 00	340 00	450 00	600 00

DETAILED INFORMATION UPON REQUEST

WILL CORPORATION

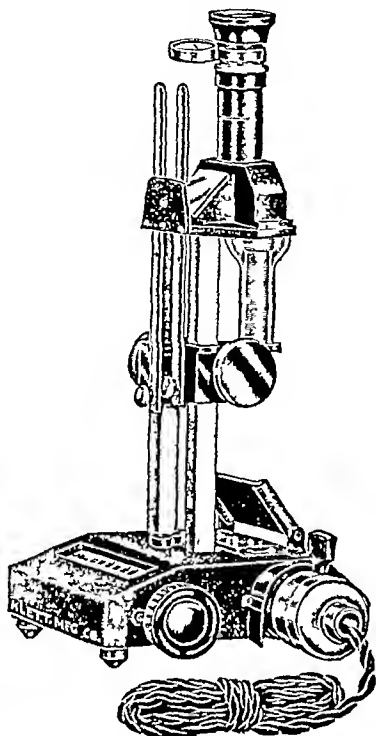
Products for Every Laboratory
Guaranteed Without Reservation

ROCHESTER, N.Y.

THE-KLETT-BIO COLORIMETER

With Permanent Gloss Standards

Patent Pending—Pat Applied for



7 POINTS OF MERIT

- 1 Color comparison, scale reading and result table can be observed under magnification from top of instrument by means of a lens with two different foci
- 2 Both stages are moved by rack and pinion with spring action
- 3 Equipped with the well-known Klett top reader, easy setting of zero point
- 4 Substage lamp controlled by switch giving a daylight effect, also equipped with a reflector for daylight
- 5 Revolving result table ranges from 6 mm to 36 mm at 15 and 20 mm standard in $\frac{1}{10}$ millimeters, also has separate table for the Newcomer hemoglobin standard with directions for its use
- 6 Table is illuminated by substage lamp
- 7 Cups and plungers resist any solvent, being directly fused, not cemented

See Article by

DR G A KILLIAN *Journal of Laboratory and Clinical Medicine* April 1925

According to authorities connected with the best institutions of the country it is considered the most convenient colorimeter made so far

The price is \$65.00 without Newcomer Standard

Klett Mfg. Co., Inc.

202 E 46th St

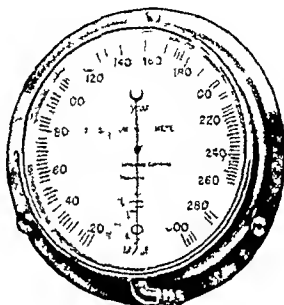
NEW YORK, N Y, U S A

AND SOLD THROUGH THE LEADING LABORATORY SUPPLY HOUSES

Mention this Journal when writing to Advertisers

Tycos

OFFICE TYPE SPHYGMOMANOMETERS



are capable of rendering invaluable service in the laboratories of Pharmacology, Physiology and Psychology Will show clearly and accurately the rate rhythm and amplitude of the pulse Gives precise determination of blood pressure

Humidity and the Physician
For Your Library
Status of the Sphygmomanometer
Blood Pressure and Surgery
Blood Pressure
Bibliography on Blood Pressure and
History of Sphygmomanometer

Copies free on request

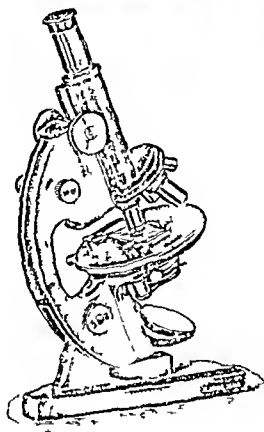
Taylor Instrument Companies

ROCHESTER N Y U S A

Canadian Plant
Tycos Building
Toronto

Manufacturing Distributors
in Great Britain
Short & Mason Ltd London

ZEISS



MICROSCOPE ESA

A Convenient and Reliable Laboratory Instrument

Price in cabinet \$132.00 f.o.b. New York

Mechanical Equipment	Simplified Mechanical Stage 12 cm diameter, Quadruple Nose piece Fine Adjustment with Graduated Drum Rack and Pinion Condenser Movement	Optical Equipment	Condenser n a 12 with Iris Achromatic Objectives 8 n a 0 20 40 n a 0 6, 90 n a 125 (Oil Immersion) Huygens Oculars 7x and 10x Magnifications 56x to 900x
----------------------	---	-------------------	--

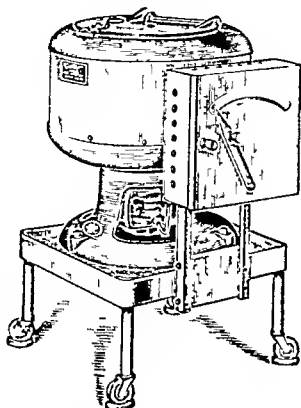
Model ESC with Square Stage and Triple Nosepiece..... \$18.00

CARL ZEISS, INC
485 Fifth Avenue
New York



Pacific Coast Branch 728 So Hill St Los Angeles

INTERNATIONAL CENTRIFUGES



PORTABLE
VIBRATIONLESS
STAND
ENCLOSED
ELECTRIC
INSTALLATION

Relative Centrifugal Forces

200 to 3800 x gravity

CAPACITIES

2 tubes of 15 ml each to
10 cups of 1500 ml each

International Equipment Co.
352 WESTERN AVENUE
Boston, Mass

THE STANDARD LOESER'S INTRAVENOUS SOLUTIONS CERTIFIED



A SYMPTOMATIC TREATMENT OF TUBERCULOSIS

Send for A Copy

A clear and concise outline of the status of tuberculosis therapy with a new viewpoint on the pharmacology of calcium and guaiacol and their effect on temperature and night sweats, showing the effect to be similar to that produced by rest in favoring the desired quiescent state so essential in the treatment of tuberculosis. Reprint and clinical data from the official publication of the Mexican Department of Military Medicine

Clinical Reports, Reprints, Price List, and the "Journal of Intravenous Therapy" will be sent to any physician on request

LOESER LABORATORY
[NEW YORK INTRAVENOUS LABORATORY]
22 WEST 26th STREET NEW YORK, N Y
Producing ethical intravenous solutions for the medical profession exclusively

Mention this Journal when writing to Advertisers

Munktell's Swedish Filter Papers



Munktell's Swedish filter paper has been on the market for many years, and is so favorably known that it is almost unnecessary to describe it. Its reputation is such that it may be said that it has become the standard by which other papers are measured, grade for grade.

J. H. Munktell makes many different types of filter paper, in order to be able to furnish a paper suitable to a large number of uses. These varieties cover the entire field of usefulness between the highest grade of analytical work and the uses calling for less expensive papers for general purposes.

We shall be pleased to correspond with anybody regarding these papers, and to furnish samples and literature giving the ash and other properties of the paper.

(1940a)



E. H. SARGENT & CO.

155 165 East Superior Street CHICAGO ILL.
LABORATORY SUPPLIES

This Journal stands for the very best in Advertising Ethics.

SOME OBSERVATIONS ON THE USE OF FRESH YEAST IN GYNECOLOGICAL PRACTICE

IN harmony with the modern view that intestinal disturbances are the source, directly or indirectly, of a host of ills once attributed to other and not infrequently obscure causes, is the opinion of an eminent medical authority on the relation of constipation to diseases of women

"It may be said," declares this authority, "that many of the distresses from which women suffer should be attributed to the intestinal toxemia, colitis and other colonic infections which are the result of constipation rather than to disease of the sex organs. This is especially true of the headaches, backaches, lassitude and general lowered vitality which are most often charged to disease of the pelvic organs and various local affections."

There are gratifying evidences that this common-sense view is today meeting with growing acceptance on the part of the medical profession. Among these evidences is the widespread use now being made of fresh yeast as a food in gynecological practice.

Women, it is well known, are more subject to constipation than men. Their lives are generally more sedentary. Their diet often fails to provide needed bulk.

And in pregnancy, of course, constipation is a constant problem.

Throughout this trying period, as well as during the ensuing lying-in period, the need is for a safe, non-irritating, non-weakening but efficient regulator of the bowel move-

ments. This need, as many thousands of physicians have discovered, is fully supplied by the ingestion of three cakes daily of fresh yeast.

Yeast gently stimulates peristalsis by increasing the bulk and moisture of the fecal masses. It also combats the intestinal toxemia resulting from prolonged putrefaction in the colon. Whereas cathartics weaken the ability of the intestine to destroy the hostile, non-acid-forming types of bacteria, yeast has an exactly opposite effect.

By aiding the colon to empty itself frequently, yeast counteracts the "reverse peristalsis" which is often declared to be the cause of indigestion and nausea. The patient's appetite is increased, and with it her general sense of well-being.

Where all that is needed is a mild tonic, yeast is also indicated. The systemic effect of fresh yeast is well known.

Physicians usually suggest three cakes daily, one before each meal. Yeast may be eaten plain or with a sprinkle of salt, spread on crackers, or suspended in milk or water. For constipation it is most effective when taken in hot (not scalding) water, one cake before each meal and at bedtime.

A copy of the latest brochure on yeast therapy, containing a bibliography of articles and references on the subject, will gladly be mailed on your request. The Fleischmann Company, Dept. 292, 701 Washington Street, New York City.

U S BUREAU OF STANDARDS

CERTIFICATION OF

BLOOD COUNTING CHAMBERS

In an article on *Blood Testing* in *The United States Daily* December 24th 1906
Dr Lewis V Judson Chief of the Length Section Weights and Measures Division U S
Bureau of Standards states

The Bureau of Standards is not engaged in any researches or testing regarding the use of the chambers in actual blood counting. It has wisely limited its activity in connection with these instruments solely to the standardization and test of their dimensions on the accuracy of which the precision of the instruments depends. It is from this point of view also that the Bureau has pointed out the advantages of the Improved Neubauer ruling and of the modern one-piece construction of the slides both being features which specialists in the medical profession also commend.

The test of haemocytometer apparatus at the Bureau of Standards undoubtedly has raised the general standard of accuracy of these instruments.

Not all manufacturers have yet reached the goal of accuracy which one of them has maintained from the beginning of work on a production basis namely a perfect score in the accuracy of counting chambers tested at the Bureau. Chambers with errors in depth amounting to as much as 10 to 20 percent have been received at the Bureau.

Some chambers with very large errors in depth have been tested within the past year. Precise blood-counting apparatus is however being made on a comparatively large production basis in this country and is available on the market. A test at the Bureau of Standards will determine the precision of apparatus submitted to it for test.

It is our opinion that the perfect score mentioned refers to Levy and Levy Hauser Counting Chambers. However we emphasize the importance of purchasing counting chambers with U S Bureau of Standards certification as such certification subsequent to purchase involves both delay and inconvenience.

If a Levy or Levy Hauser Counting Chamber purchased without certification should fail to pass Bureau of Standards certification it will be replaced by us without charge. No such instance has occurred in the sale of over 45 000 Counting Chambers.

The certification of counting chambers and diluting pipettes by the U S Bureau of Standards was first undertaken in 1917 at our request.

For detailed description and price list of Levy and Levy Hauser Chambers see pamphlet EE 26 copy of which is sent upon request

ARTHUR H THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE

PHILADELPHIA U S A

CABLE ADDRESS BALANCE PHILADELPHIA

REPORT OF A PRACTICAL TEST

ON

STRAIN-FINDER TESTED TEST TUBES

The chief of the Division of Agricultural Biochemistry, of the Department of Agriculture of one of our largest State Universities, writes as follows

May 28th, 1927

"I have your letter of the 21st, together with a package of strain-tested test tubes

"I had one of our boys test these out by adding 20 cc of ice water and then immediately sticking the test tube in the hottest part of a Meker flame. Under less drastic conditions, we have in the past been able to save about one test tube out of three. Using your tubes, one tube broke on the first trial, the other eleven came through the first trial successfully and, on repeating the experiment, all eleven came through a second time. This is somewhat more drastic treatment than a test tube is usually put up against, and I would say from this experiment that the tubes are highly satisfactory."

In reply to our request for permission to publish the above report, this Professor writes

June 15th, 1927

"I will be perfectly willing for you to send out that information because, as I told you, I have been thoroughly disgusted in the past with _____ test tubes and have wasted a great deal of valuable material by the breaking of test tubes just at the time that I did not want them to break."

For prices, packing and discounts for large quantities, with details of the strain-finder test, see supplement No 83, copy of which is sent upon request

Sole Distributors

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE

PHILADELPHIA, U S A

CABLE ADDRESS 'BALANCE PHILADELPHIA

American Museum Jars in many sizes

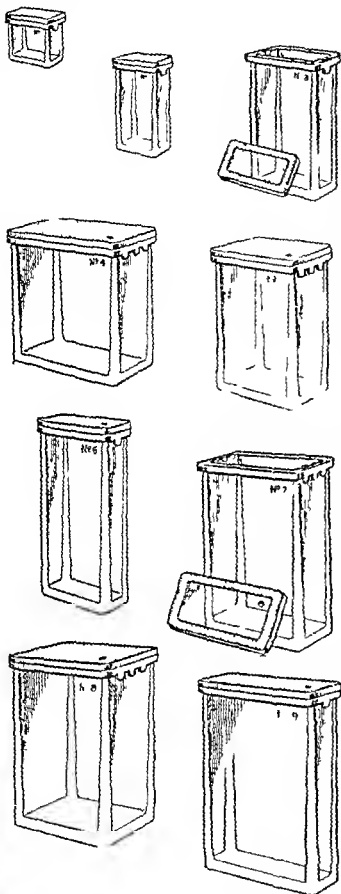
These American made jars have qualities which place them far ahead of the imported jars offered in previous years. They are not blown but pressed in iron molds assuring uniform thickness of walls. This prevents distortion of the specimen and the use of decolorized glass also aids in presenting the specimen in its true appearance.

Suspension rods may be laid across the recesses at the top of the jars and specimens are thus hung in the fluid. This avoids the necessity of clumsy pedestal supports.

The covers which are pressed from the same clear glass each contain a small opening which acts as a vent and permits addition of preserving fluid after sealing.

The International Association of Medical museums has recommended the various sizes which are furnished. No. 1 is 5x5x3 cms and the largest is 25x25x12 cms.

For details and prices consult our catalogue



FISHER

SCIENTIFIC COMPANY

Laboratory Apparatus and Reagents for Chemistry, Metallurgy, Biology

PITTSBURGH, PA

IN CANADA, FISHER SCIENTIFIC CO. LTD., 472 MCGILL ST., MONTREAL

NATIONAL BIOLOGICAL DYES

NATIONAL Biological Dyes and Indicators have earned an outstanding reputation for quality, uniformity and dependability. They are today the choice of an increasing number of educational institutions, hospitals, pathologists and commercial laboratories.

The National line includes by far the largest list of Commission Certified Stains offered by any manufacturer.

Stocked by the principal dealers in laboratory supplies.

Complete catalog and price list on request.

Pharmaceutical Laboratories

NATIONAL ANILINE & CHEMICAL COMPANY, Inc.
40 Rector Street New York City



Superior Products to combat

DIPHTHERIA

Super Concentrated Diphtheria Antitoxin

Prevention

For curative treatment and immunization of contacts

An antitoxin of very low protein content small bulk highly refined which minimizes serum sickness and is rapidly absorbed

Less bulk—Less pain—Quicker results

Treatment

DOSAGE—1000 3000 5000 10 000 20 000 units

Supplied in Mulford Perfected Syringes ready for immediate use

Diphtheria Toxin Antitoxin Mixture

Susceptibility

To produce a lasting immunity, which develops in about eight weeks and continues effective for some years probably for life

Immunization of children of pre school age and up to 10 years is recommended

DOSAGE—3 injections of 1 cc each at 7 day intervals

Convenient Packages

Supplied in packages containing

Super Quality

3-1 cc vials (1 immunization)
30-1 cc vials (10 immunizations)
10 cc vials (3 immunizations)
30 cc vials (10 immunizations)



Schick Test Toxin

To determine susceptibility to diphtheria

DOSAGE—0.1 cc injected between the layers of the skin

Supplied in packages containing sufficient material for 50 tests.

H K MULFORD COMPANY

PHILADELPHIA U S A

7573

Mulford

THE PIONEER BIOLOGICAL LABORATORIES

Successful Practice

Correct diagnosis, proper treatment and the wise selection of drugs are three essentials of successful practice. The following remedies may be depended upon in the conditions indicated. They lend themselves to successful practice.

ASTHMA

EPHEDRINE HYDROCHLORIDE

is reported in medical literature to be giving excellent results in asthma. The Abbott brand of guaranteed purity is supplied in tablets and capsules for hypodermic or oral administration, in 3% solution for topical use and also in powder form and as a nasal spray with Butesin.

RHEUMATISM

NEOCINCHOPHEN

The wide use of this drug in rheumatism, sciatica, gout, lumbago and neuralgias stamps it as an outstanding success. The reasons for its action are well-founded and the results justify its selection in these cases. NEOCINCHOPHEN, ABBOTT, is the choice of discerning physicians.

COLDS

METAPHEN

NEUTRAL
ACRIFLAVINE
NEONAL
BUTYN
BUTESIN
PICRATE
OINTMENT
CHLORAZENE

The intra-nasal use of Metaphen sprays in early colds is doing much to shorten their course and prevent more serious complications. METAPHEN is more than an ordinary germicide. It is a real addition to the doctor's weapons of offense and defense against invading and destructive organisms.

*These and Other Abbott and D R L Preparations May be Obtained
of Your Druggist or Local Dealer*

The ABBOTT LABORATORIES

North Chicago, Illinois

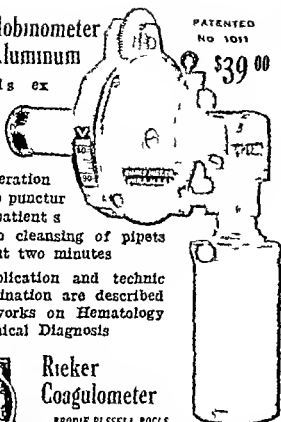
New York San Francisco Seattle Los Angeles Toronto Bombay

Hemoglobinometer Dare Aluminum

Blood is examined undiluted. Complete operation from the puncturing of patient's finger to cleansing of pipets takes but two minutes

The application and technic of examination are described in all works on Hematology and Clinical Diagnosis

PATENTED
NO 1011
\$39 00



Rieker Coagulometer

BRODIE RUSSELL ROGGS
PATTERN

Most accurate and quickest method for determining coagulative quality of the blood

Write for descriptive circulars



For sale by H
Supply H men

RIEKER INSTRUMENT COMPANY
1919 Fairmount Avenue Philadelphia Pa U S A

The outstanding reason for the universal satisfaction found in DAIGGER LABORATORY EQUIPMENT is summed up in one word

CARE

Care in adherence to specifications
Care to be prompt in shipment
Care in packing

Send for a copy of our
catalog

ESTABLISHED 1894

A. Daigger & Co

Laboratory Supplies and Chemicals
Kearney & Wabash Sts CHICAGO ILL.



For Urinary Analysis

Clean
Odorless
Quick
Accurate

NO 910
\$2.00

There is no handling of wet
specimens when retested
made with this outfit. It is
easy to use. Add the liquid
reagent on the float glass and
the result is read. Only a small
specimen is needed. Saves a lot
of time and trouble.

Testrite Inst Co 186 E 16th St N Y C

Mansfield Powders and Cosmetics

are successfully used by patients suffering from asthma and kindred ailments. Recommended by the leading physicians specializing in the treatment of these diseases

Write for formal samples to the

E R Mansfield Laboratories
1949 1/2 S. Los Angeles St Los Angeles California

GUINEA PIGS RABBITS-MICE

Are You in Need

of Guinea pigs rabbits
white mice white rats
cats or dogs?

If prompt delivery of
efficient service and good
quality stock is a con-
sideration when buy-
ing ask for my prices.

Wholesale distributor of
Laboratory Animals
since 1912



E MICHAELS
2907 DIAMOND ST
PHILADELPHIA

Apparatus for Experimental Work and Research

For use in laboratories devoted to the study and teaching of
Pharmacology, Physiology and Related Subjects

WH 137 Kidney
Oncometer

WH 130 Myocardio
graph

WH 144 Spleen
Oncometer

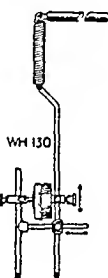
WH 200 Adjusta
Pulley

WH 207 Arterial
Canula

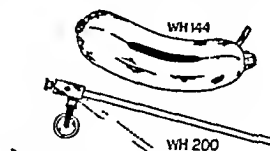
WH 214 Pulmonary
Arterial
Canula



WH 137



WH 130



WH 144



WH 207



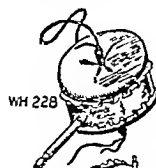
WH 214



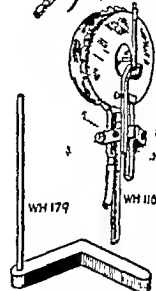
WH 165



WH 186



WH 228



WH 179

WH 165 Lung
Plethysmo
graph

WH 186 Hart Lever

WH 228 Stethograph
Drum

WH 179 Supporting
Stand

WH 116 Tamhour
Single

We specialize in the complete equipment of laboratories for Medical Schools Hospitals and Research Institutions
Part of our large factory is devoted to the manufacture of Experimental and Research Apparatus

THE MAX WOCHER & SON CO.

Surgical Instruments, Chemical Glassware, Hospital and Laboratory Furniture

29 31 West Sixth St

Cincinnati, O

Erythrol Tetranitrate Merck

Literature on request

Effective Vasodilator

Useful in Angina Pectoris,
vascular diseases, and as
a prophylactic for anginal
pain

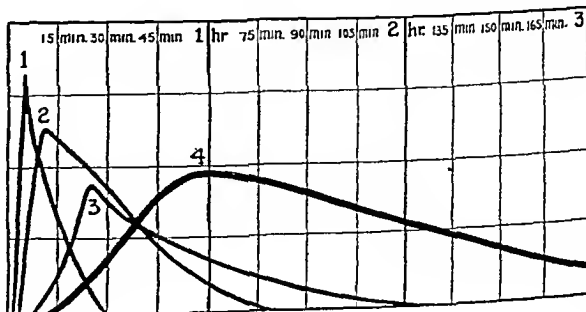
Tablets— $\frac{1}{4}$ grn Bottles of 50

Tablets— $\frac{1}{2}$ grn Tubes of 24
and Bottles of 100

Chart shows relative re
duction of pulse tension
produced by

- 1 Amyl Nitrite
- 2 Nitroglycerin
- 3 Sodium Nitrite
- 4 Erythrol Tetranitrate

MERCK & CO.
INC
Rahway, N J



"AMPOULES, PREFERRED"

As a Therapeutic Investment

A physician writes us

"For several years I have been gradually eliminating from my practice the uncertainty of self-medication by my patients and relying more and more upon treatments with known quantities of the drugs I desire in ampoule form.

The forgetfulness and seeming indifference of patients even with the most carefully given instructions for the use of prescribed drugs frequently interfered with expected improvement.

The theory of ampoule medication appealed to me through its complete control over dosage and administration and also because of its quicker action, purity and standardization.

Since using ampoule medication I note an increased efficiency particularly in instances where oral administrations cannot be tolerated because of digestive disturbances.

My patients improve faster and except in a few isolated instances among timid or hypersensitive patients who object to the needle I find that they are better satisfied with the results."

Breon Ampoule Solutions will likewise benefit *your* practice.

We will gladly send descriptive literature. Just mail this coupon to any of the addresses below.

GEORGE A. BREON & CO

Pharmaceutical Chemists

Kansas City, Mo.

New York
152 W. 42nd St.

Chicago
1015 So. Wabash

Atlanta
100 Rhodes Bldg.



FROM NOW ON,—
USE BREON!

George A. Breon & Co., Kansas City, Mo.

Please send me descriptive literature and full information on ampoule medication.

Signature of Practitioner

Dr.

Street

City

State

The Pottenger Sanatorium

MONROVIA, CALIFORNIA

**FOR DISEASES OF THE
LUNGS AND THROAT**

A thoroughly equipped institution for the scientific treatment of tuberculosis

High class accommodations

Ideal all year round climate Surrounded by orange groves and beautiful mountain scenery

Forty five minutes from Los Angeles

F M Pottenger A M M D L L D Medical Director

J E Pottenger A B M D Assistant Medical Director and Chief of Laboratory

We have established in connection with the Sanatorium a clinic for the diagnosis and study of such non tuberculous diseases as asthma lung abscess and bronchiectasis

For particulars address

Pottenger Sanatorium

Monrovia California

LOS ANGELES OFFICE

1045 47 Title Insurance Building

New 2nd Edition

Examination of Children

By Clinical and Laboratory Methods

By A Levinson, B S, M D, Associate in Pediatrics Northwestern University Medical School Attending Physician Children's Dept Cook County Hospital Attending Pediatrician Sarah Morris Hospital for Children of the Michael Reese Hospital Chicago etc

192 pages 7x10 with 85 text illustrations and color plates Second Edition revised and enlarged Price cloth \$3 50

Includes all the procedures that a pediatrician or general practitioner who comes in contact with children has to carry out in his practice Only the most simple tests that can be performed by the average physician are described in the book All complicated tests are discussed with reference to interpretation with particular reference to their application in pediatric practice

C V Mosby Co—Publishers—St Louis

“Good Things to Come”

Static Electricity in the Treatment of Pelvic Diseases William Benham Snow New York City

The Vascular Relaxation of the Splanchnic Area Mary Arnold Snow New York City

Clinic on Internal Medicine Hilding Berglund Minneapolis Minn

Influence of Rectal Disease Upon the General Health Charles J Drueck Chicago Ill

Bilateral Hydronephrosis Case Reports Julius Frischer and Frederick E Diemer Kansas City Mo

Late Results of Upper Extremity Reconstruction Work A Steindler Iowa City Iowa

Diathermy in Painful Shoulder Conditions

Richard Kovacs New York

Hepatic Stasis and Its Associated Conditions

William Martin Atlantic City

Treatment of Deep Seated Pain With Physical Therapy Benjamin H Sherman Dexter Iowa

The Quick and the Dead Burton Baker Grover Colorado Springs

Report of Two Unusual Cases of Ascites Relieved by Diathermia A F Tyler Omaha Neb

The Various Electrotherapy Modalities and Their Special Indications, Jos E G Waddington Detroit Mich

Honesty in the Profession L A Martz Kansas City Mo

Is Physical Therapy a Rational Procedure?

L A Poyntz, Chicago Ill

The Use of Fulguration and Electric Coagulation in Treatment of Bladder Tumors Clinton K Smith Kansas City Mo

The Therapeutics of Hyperpyrexia With Special Reference to Infra Red Radiation G J Warmuth Milton Wis

The Use of the Low Voltage Currents in Gynecology A David Willmoth Louisville Ky

Physiotherapy in Practice A David Willmoth Louisville Ky

Chronic Arthritis With Obesity S Grover Burnett Kansas City Mo

Static Electricity as a Systemic Tonic, J D Gibson Denver Colorado

The Leucocyte Count as a Diagnostic and Prognostic Aid in Appendicitis M Pinson Neal and Dudley A Robnett Columbia Mo

All of the above excellent papers and many more will appear shortly in

The Medical Herald and Physiotherapist

Only Two Dollars The Year

Contains all the Transactions of the Medical Society of the Missouri Valley and the Western Assn of Physical Therapy

CHAS WOOD FASSETT, M D

Managing Editor

115 E 31st Street

Kansas City, Mo

Reports of American Physicians

This pamphlet relates their observations with some typical cases in which they prescribed Tolysin

May we send you a copy?

THE CALCO CHEMICAL CO , Bound Brook, N J


In Gastric Ulcer

Hare and others have drawn attention to the persistent presence of an excess of hydrochloric acid both as to percentage strength and quantity

Kalak Water helps to combat such hyper acidity It is unusually well borne and preferable to single alkalies because less apt to set up an alkalosis

KALAK WATER CO , 6 Church St New York City


**HOTEL
BALTIMORE**
KANSAS CITY



500 ROOMS

ROOM TARIFFS	MARBLE COFFEE SHOP
Room without Bath - \$2.00 and up	Table D'Hote LUNCH ~ 75¢
Room with Bath - \$2.50 and up	DINNER - \$1.25 & 1/2
Double Room with Bath - \$4.00 and up	A la Carte Service

**HOTEL
Muehlebach**



KANSAS CITY

An outstanding hotel of the middle-west
offering every service demanded by the
most exacting guest.

Daily Amusements
Music and Dancing - Plantation Grill
Famous Trianon Cafe
Also Coffee Shop for Quick Service

BOOK BINDERS

We specialize in the binding of new publications and the rebinding of old books. Also in Edition or Catalog binding in cloth or leather and Pamphlet work

Becktold Company

200-212 Pine St

Established 1872

St Louis, Mo

Just Published

Gonococcal Infection in the Male

By Abr L Wolbarst, M D

Urologist and Director of Urologic Clinics Beth Israel Hospital Consulting Urologist
Central Islip State Hospital Manhattan State Hospital and Jewish Memorial
Hospital etc

With a Chapter written by J E R McDonagh F.R.C.S. Surgeon London Lock Hospital
Late Hunterian Professor Royal College of Surgeons, etc London

237 pages 6x9 with 89 illustrations including seven color plates
Price cloth \$5.50

TABLE OF CONTENTS

I Introduction	XI Chronic Prostatitis
II Anatomy	XII Chronic Seminal Vesiculitis
III Acute Gonococcal Urethritis Acute Gonorrhea	XIII Diathermy in Gonorrhea
IV Acute Gonorrhea	XIV Methods of Determining the Source of Pus and Shreds in the Urine
V Diagnosis	XV When is Gonorrhea Cured?
VI Treatment of Acute Uncomplicated Gonorrhea	XVI Sexual Neuroses Following Gonor rhea
VII Complications of Acute Gonorrhea	XVII Male Sterility Following Gonorrhea
VIII Chronic Gonorrhea	XVIII Personal Prophylaxis
IX Stricture of the Urethra	XIX Gonorrhea in Male Children
X Chronic Posterior Gonorrhea	XX Views on Gonorrhea By J E R McDonagh F.R.C.S. London

PUBLISHER'S ANNOUNCEMENT

This is a highly practical work intended for the general practitioner of medicine. Not a pocket syllabus, but a work that will meet the needs of the practitioner, giving him everything he needs in diagnosis and treatment of gonorrhea and its complications. It is right up to the minute and includes a chapter on diathermy, one on the cure of gonorrhea, and all the latest tests, gonorrhea in boys, personal prophylaxis, and a special chapter by Dr McDonagh of London, giving his views on gonorrhea and its treatment. Beautifully illustrated with text illustrations and seven color plates.

THE O V MOSBY COMPANY—Medical Publishers—St. Louis

Gentlemen: Please send me a copy of the new book by Wolbarst on GONOCOCCAL INFECTION IN THE MALE. Price \$5.50 ☐ I enclose herewith check for \$5.50 ☐ Charge to my account.

Name _____ Address _____

(16 Jour)

JAMES Y SIMPSON, M D
Neurologist and Addictologist

HERMON S MAJOR, M D
Neuro-psychiatrist

SIMPSON-MAJOR SANITARIUM

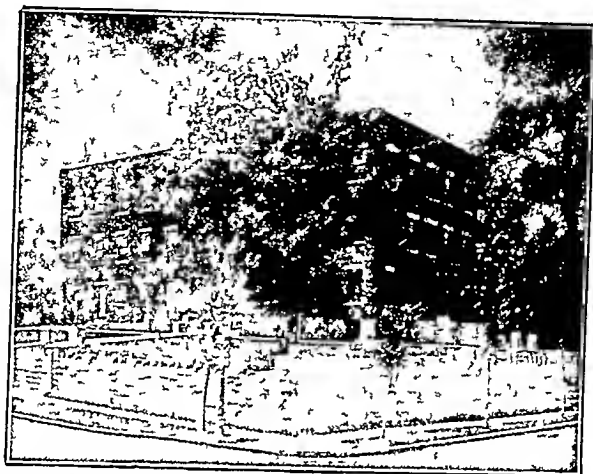
3100 Euclid Avenue

Kansas City, Missouri

Nervous
Diseases

Selected
Mental
Cases

Alcohol
Drug and
Tobacco
Addictions



Electricity
Heat
Water
Light
Exercise
Massage
Rest
Diet
Medicine

Beautifully situated in a pleasant residence section of the city Fully equipped and well heated All pleasant outside rooms Large lawn and open and closed porches for exercises Experienced and humane attendants Liberal, nourishing diet Resident physician in attendance day and night

New York's Newest!

HOTEL MANGER

Seventh Avenue and Fiftieth Street, New York City

1750 ROOMS

The Wonder Hotel of New York
A Modern, Marble Palace

ONE of the largest and finest hotels in the world, with rates lower than any other first class hotel in the country In the heart of the Times Square District—grouped about the hotel are the largest legitimate, vaudeville and motion picture theatres, as well as important shops and the New Madison Square Garden The elegant furnishings set a precedent in the equipping of a distinctive, modern hotel. Subway and surface lines at door bring Grand Central and Pennsylvania Stations within easy access

All rooms have hot and cold running water, circulating ice water and Servidors

RATES PER DAY—NONE HIGHER

	For One Person	For Two Persons
Rooms	\$2 50—\$3 00	\$3 50—\$4 00
Rooms with shower bath	3 00—3 50	4 50—5 00
Rooms with private bath and shower	3 50—4 00	5 00—6 00

OWNERSHIP MANAGEMENT



MONKEYS

FOR LABORATORY RESEARCH

Macacus Rhesus

(Small and Medium)

also other Genera

HENRY BARTELS

72 74 Cortlandt St

New York City

Write for complete list with prices

Third Edition Revised and Enlarged Now Ready

Compendium of Regional Diagnosis in Affections of the Brain and Spinal Cord

A Concise Introduction to the Principles of Clinical Localization in Diseases and Injuries of the Central Nervous System

By ROBERT BING Professor in the University of Basle Translated from the Sixth German Edition by F S ARNOLD B.A. M.B. B.Ch. (Oxon)

201 Pages 6x9 102 Illustrations many in Colors Price \$17 cloth \$6.00

DIVISION I Regional Diagnosis of Brain Lesions (a) Lesions in the region of the cerebral axis or brain stem (b) Lesions of the cerebellum (c) Lesions of the cerebrum the basal ganglia and the hypophysis

DIVISION II Regional Diagnosis of

The C V Mosby Co—Publishers—St Louis, U S A

You Need These Two Journals

THE JOURNAL OF AYURVEDA

EDITED BY

MAHMAHOPADHYAYA KAVIRAJ GANANATH
SEI SARASWATI M A LMS

First issue appeared in July 1924
Copies available from August 1924 Vol
ume III began from July 1926 Annual
subscription Rs 10 Post Free The
Journal is attractively got up with clear
type and appears to be a clearing house
for progress in Ayurvedic medicines —
PRACTICAL MEDICINE

Size 8vo 40 pages reading matter
crispful of useful and interesting subjects

Sample Copy free

For Advertisement rates and special rates of
subscription for juniors and combined subscribers

APPLY TO — THE MANAGER INDIAN MEDICAL RECORD
2 Horo Kumar Tagore Square
Corporation Street East, Calcutta, India

THE INDIAN MEDICAL RECORD

Journal of Public Health & Tropical
Medicine The Medical Monthly to keep
you abreast of the progress made in trop-
ical medicine and hygiene

Eminent European and Indian con-
tributors regularly contribute to its col-
umns It contains also a summary of
current improvements in all branches of
medical science as appear in the Medical
Press of the world

Vol XLVII begins January 1927
Subscription may commence from any
month or from January 1927

Annual subscription Rs 7 8 0

Sample copies free to the profession
on application

THE CHINA MEDICAL JOURNAL

Established 1887

Official organ of

THE CHINA MEDICAL ASSOCIATION

The Leading Medical Journal of China—Monthly Price per annum
U S \$5 00, Post Paid

This Journal deals with the nosogeography of China, the symptomatology, pathology, distribution of diseases and the pharmacology and materia medica of that country

No medical library or student of world medicine can afford to disregard the nosology of an area that contains a fifth of the population of the globe

Order from THE BUSINESS MANAGER, China Medical Journal,
P. O. Box 1121, Shanghai, China

The JOURNAL OF THE PHILIPPINE ISLANDS MEDICAL ASSOCIATION

547 Herran Street

Manila

PHILIPPINE ISLANDS



Issued monthly Investigations in Tropical Medicine are published Annual subscriptions \$3 00 (U S Currency) For advertising rates apply to the Business Manager of the Journal, 547 Herran Street, Manila, Philippine Islands



Nature's Iodine Restored

As a preventive of simple goiter we are restoring the natural iodine to salt. This more complete food, containing the iodine so necessary to the daily diet, is produced under the watchful supervision of certified chemists. Each two pound package contains the exact amount of Potassium Iodide recommended by health authorities.

MORTON SALT COMPANY
CHICAGO

When It Rains—It Pours

Every library should have it and every one doing biologic work of any kind should have access to it as it contains everything he may want to know about methods of work
 of Laboratory and Clinical Medicine The Journal

ueber das

Handbuch der Biologischen Arbeitsmethoden

Unter Mitarbeit von ueber 600 Fachmaennern herausgegeben von
 Emil Abderhalden, Halle, a S

Von diesem fuer den Forscher unentbehrlichen Handbuch sind bisher 236 Lieferungen aus fast allen Abteilungen und Teilen des Werkes erschienen gebundene Baende liegen bisher 28 vor Jede Lieferung und jeder Band ist auch einzeln kaeuflich so dass es jedem ermoeglicht ist die seinem besonderen Arbeits und Forschungsgebiet entsprechende Auswahl zu treffen

Ausfuehrliche Inhaltsuebersicht auf Verlangen Kostenlos!

Mit dem soeben erfolgten Erscheinen des III Bandes liegt vollstaendig vor

Enzyklopaedie der mikroskopischen Technik

Dritte, vermehrte und verbesserte Auflage
 Unter Mitarbeit zahlreicher Fachgenossen herausgegeben von
 Prof Dr Rudolf Krause, Berlin

Mit 350 Figuren im Text 36 farbigen und 12 schwarzen Tafeln

Drei Baende mit ausfuehrlichem Sach- und Namenregister VIII III 2445 Seiten Gehftet RM 134 — gebunden RM 148 —

Kein Laboratorium in welchem mikroskopisch gearbeitet wird und alle modernen Methoden zur Hilfe herangezogen werden sollen wird auf die Anschaffung dieser neuen Auflage welche eine Riesensumme gewissenhaftester Arbeit einschliesst verzichten koennen (Anatomischer Anzeiger)

Ergebnisse der gesamten Medizin

Unter Mitwirkung hervorragender Fachgelehrten herausgegeben von
 Prof Dr Th Brugsch, Berlin

Die Ergebnisse der gesamten Medizin erscheinen in zwangloser Folge zur Zeit gelangt Band 1 zur Ausgabe Zahlreiche Textbilder und Tafeln sind beigegeben Jeder Band kostet bei einem Umfang von etwa 600 Seiten gebunden gegen RM 30 —

Those who understand German enough to follow a medical text will find in this periodical a fund of knowledge not obtainable in ordinary or regular periodicals (Clinical Medicine)

Verlag von Urban & Schwarzenberg / Berlin und Wien

Die Lieferung dieser Werke wie auch aller anderen deutschen Bucher, erfolgt—sofern nicht anders erwuunscht—durch

Oscar Rothacker Buchhandlung und Antiquariat

Berlin N 24, Friedrichstr 105 B

Evolution of Preventive Medicine

By Sir Arthur Newsholme, KCB, MD, FRCP

The former Principal Medical Officer of the Local Government Board of England presents the first concise and authoritative narrative of the development of public health work from ancient times to modern. Cloth, 5 x 7 1/4, 226 pages, 6 illustrations, index. Price \$3 00

Immunity in Syphilis

By Alan M Chesney, MD, Johns Hopkins Medical School

In this monograph Dr Chesney presents a critical review of the contributions to the study of immunity in syphilis—more particularly those of recent years—but not neglecting the historical aspects of the subject. Cloth, 6 x 9, 85 pages, 163 references. Price \$2 50

Serum Diagnosis of Syphilis by Precipitation

By R L Kahn, Michigan Department of Health

Presents the governing principles, procedure and clinical application of the Kahn Precipitation Test. Cloth, 6 x 9, 237 pages, index, bibliography. Price \$3 00

Standard Methods of the Division of Laboratories and Research of the New York State Department of Health

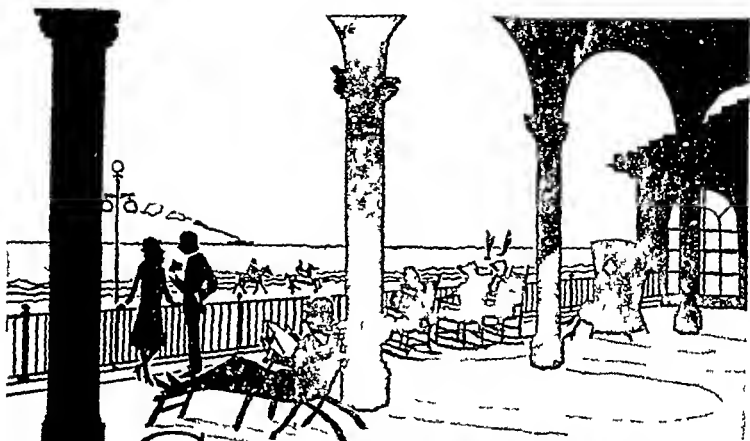
By Augustus B Wadsworth, Director

A comprehensive guide to general laboratory procedures and methods used in the department for preparing media and glassware, the laboratories for sanitary and analytical chemistry, the research, publications, and library department, the anti-toxin, serum, and vaccine laboratories, the diagnostic laboratories, and the executive offices. Cloth, 6 x 9, 646 pages, 70 illustrations, index, references. Price \$7 50

THE WILLIAMS AND WILKINS COMPANY

Publishers of Scientific Books and Periodicals

Baltimore, Maryland



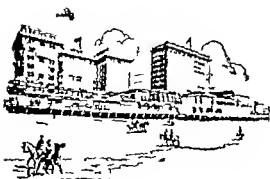
CHALFONTE~ HADDON HALL ATLANTIC CITY

*In the very center of things
on the Beach
and the Boardwalk*

*Dual Trio Radio Concert
every Tuesday evening—
Tune in on WPG at 9*

STAND out like personal friends in the thoughts of those who love to go down to the sea for rest or play—their simple friendly hospitality has so graced every service for so many years

Delightful during the fall and winter months are the broad deck porches facing the sea with their comfortable steamer chairs looking down on the flowing life of the Boardwalk. For the more active—golf riding on the beach theatres Boardwalk activities fascinating shops music and entertainment



American Plan Only Always Open

Illustrated Fold on Request

LEEDS AND LIPPINCOTT COMPANY

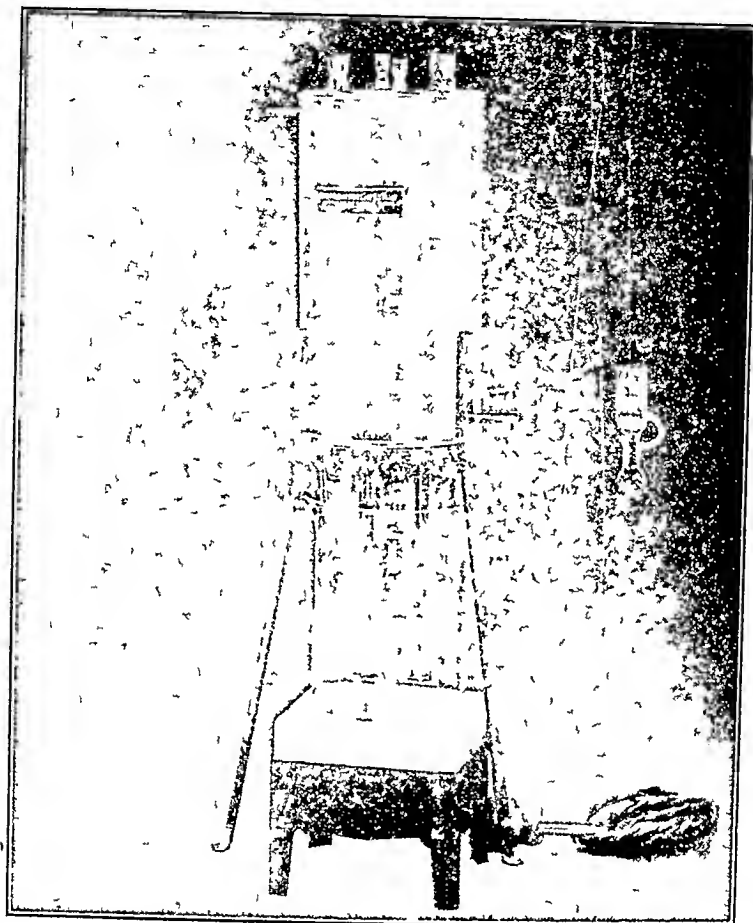
INDEX TO ADVERTISERS

Please mention 'The Journal of Laboratory and Clinical Medicine' when writing to advertisers — It identifies you

Abbott Laboratories -----	24	Kalak Water Co -----	9
Baltimore Muehlebach Hotels Kan sas City -----	0	Kewaunee Mfg Company --	14
Bartels, Henry -----	3	Kimble Glass Co -----	Insert
Bausch & Lomb Optical Co -----	10	Klett Mfg Co Inc -----	12
Becktold Company -----	30	LaMotte Chemical Products Co -----	3
Becton, Dickinson & Co -----	7	Loefer Laboratory (N Y Intrav Laby) -----	16
Breon & Co, Geo A -----	9	Manger Hotel New York City --	3
Calco Chemical Company -----	9	Mansfield Laboratories E R -----	29
Calcutta Medical Journal --	39	Medical Herald and Physiotherapist	8
Cambridge Instrument Co Inc	11	Merck & Company Inc -----	6
Castle Co, Wilmot -----	9	Miehals F -----	5
Central Scientific Company --	6	Morton Salt Co -----	30
Chalfonte Haddon Hall Atlantic City -----	41	Mulford Company H K -----	2
China Medical Journal -----	34	National Aniline & Chemical Co --	2
Collins, Warren E -----	39	Palo Company --	15
Comer, Harris (Steele Glass Co) -----	3rd cover	Pfaltz & Bauer Inc -----	3
Daggor & Co A -----	25	Puttenger Sanatorium -----	28
Dean Laboratories --	10	Reig & Co D Domenech -----	39
Digestive Ferments Co --	1	Rickert Instrument Company --	3
Doniger & Company S -----	7	Sargeant & Co F H -----	1
Eimer & Amend -----	4th cover	Schaar & Co -----	2
Empire Laboratory Supply Company	44	Simpson Major Sanitarium --	32
Fairchild Bros & Foster -----	9	Swan Myers Company -----	8
Fales Chemical Co Inc -----	18	Taylor Instrument Company -----	12
Fisher Scientific Company --	21	Testrite Instrument Co -----	25
Fleischmann Company -----	20	Thermo Electric Instrument Co -----	2nd cover
Gradwohl Laboratories --	2	Thomas Company Arthur H -----	Insert
Harris Laboratories Inc -----	Insert	Toledo Technical Apparatus Co --	38
Harrower Laboratory Inc --	4	Verlag Urban & Schwarzberg -----	36 37
Indian Medical Record -----	33	Will Corporation -----	11
International Equipment Co -----	16	Withams & Wilkins Co -----	40
Journal of Philippine Islands Med ical Ass'n -----	34	Woehner & Son Max -----	26
		Zeiss Inc Carl -----	13

LEIBOFF CHOLESTEROL APPARATUS

Patent applied for



This apparatus was designed for Cholesterol determination in blood and is an improvement on the present method for the following reasons

Uses Smaller Amounts of Blood

($\frac{1}{4}$ of the usual amount)

Saves considerable labor and time. The complete test may be made in about 40 minutes also there is no loss of material since no transfer of material is made the whole procedure is done in one tube for this reason the method is more accurate

The apparatus is portable and very compact occupying very little space in the laboratory. One of the most important features is that six simultaneous extractions can be made with one condenser also it cannot leak and parts can be easily replaced

Directions for use supplied with apparatus

No 6173	Cholesterol condenser complete with glass tubes and 6 ft rubber tubing but without heater filter discs or beaker	\$35 00
No 6173/A	Plain glass condenser tubes	each 60
No 6173/B	Special Leiboff tube mark at 5 ml	each 1 00
No 6173/C	Specially prepared filter disc (Package of 100)	2 50
No 6173/D	Electric heater with plug and cord	6 25

(State Current & Voltage Required)

Reference—(Journal Biological Chemistry Vol LXI No 1 Aug 1924 page 177)

Sold Exclusively by

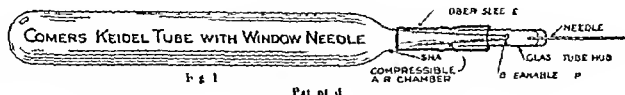
EMPIRE LABORATORY SUPPLY CO.

Incorporated

Importers, Manufacturers and Dealers

Chemical Apparatus, Chemicals and Bacteriological Supplies

218-20 East 37th Street, New York



Comer's Window Needle *makes it possible to SEE that*

The breakable tip of ampoule is not prematurely broken
The Blood shows in Glass Hub when needle is properly introduced into vein

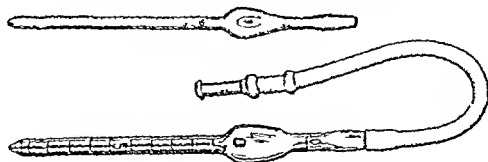
This eliminates the sight unseen features of the Original Keidel Tube and will make much more certain the obtaining of good blood samples

We offer also—

BLOOD CHEMISTRY KEIDELS

with Sodium Fluoride as used by the Pennsylvania
Department of Health under the direction
of Dr John L Laird

Comer This trade mark on the back of our Blood Count
Pipette means that we guarantee a Greater Accuracy
than is required by the U S Bureau of Standards



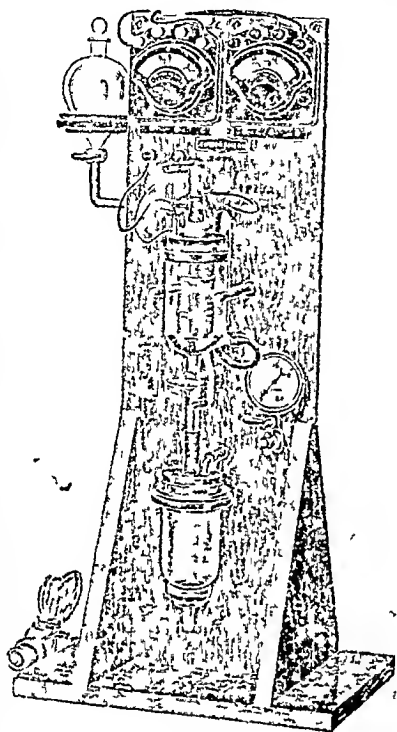
HARRIS COMER, Trading as Steele Glass Company

622 South Washington Square

PHILADELPHIA PA

We make apparatus of Glass for Chemical Medical and Experimental Laboratories
Forty Years Experience

BRONFENBRENNER APPARATUS



A Simple Electro-Ultrafilter

(See Jour. of General Physiology, Sept 20, 1926, Vol. X, No. 1, pp. 23-26)

Combines ultrafiltration with electrophoresis. Used for freeing colloidal solutions of electrolytes, dialyzing solutions containing bacteriophage, and for similar work. Capacity about 25 cc.

The author describes the performance of the apparatus as follows: "When 20 cc. of broth were placed in the dialyzing chamber and the full current was turned on the ammeter read 4.5 amperes. If this amount of broth were dialyzed without current, it would require 3 hours to increase its resistance to the passage of current sufficiently to give an ammeter reading of 0.016. When dialysis was combined

with electrophoresis, a reading of 0.013 amperes was reached in 45 minutes."

This ingenious design utilizing both the front and back of the support, results in an apparatus in which twenty-eight parts, including electrical instruments, shaking motor, etc., are combined into one complete unit ready for immediate operation on direct current.

Write for descriptive literature and prices

EIMER & AMEND

Est 1851

Inc 1897

Headquarters for Laboratory Apparatus and Chemical Reagents

Third Ave., 18th to 19th St.

NEW YORK, N. Y.

